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SURFACE-ACTIVE
QUATERNARY AMMONIUM GERMICIDES

Surface-Active Quaternary Ammonium Germicides

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FOREWORD

To the best of the author's knowledge no comprehensive review of the entire field of quaternary ammonium surface-active germicides exists in the literature. As he has worked in this field for the past decade, he has found the need for such a compilation. This has not only been reflected in his own work but also in comments and discussions with other investigators.

In preparing this review the writer is cognizant of the fact that with the most careful scrutiny of the literature important publications on this subject may be overlooked and space limitations often necessitate inadequate consideration of others. For this reason due apologies are herewith made to authors of articles on quaternary ammonium compounds of which inadequate or no mention is made in this monograph. Comments and suggestions of the reader will be welcome so that the present text can be kept up-to-date in future editions.

The author is grateful to Drs. M. L. Tainter, C. M. Suter and F. C. Nachod, of the Sterling-Winthrop Research Institute, for their helpful comments and suggestions in preparing this monograph.

CARL A. LAWRENCE

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1. Introduction

The quaternary ammonium surface-active antiseptics have secured a prominent and important place in the fields of medicinal and general disinfection during the past decade. These compounds are of particular interest in that, unlike most other disinfectants, they exhibit not only germicidal action but also surface-active, detergent, and wetting properties.

Jacobs, Heidelberger, and their co-workers at the Rockefeller Institute reported as early as 1916 on the bactericidal properties of a large number of quaternary ammonium salts of hexamethylenetetramine. However, almost 20 years elapsed before any further significant contributions to this field were published. It remained for Domagk (1935) to focus attention on surface-active quaternary ammonium compounds as germicides and antiseptics.

The number of publications on the clinical use of alkyldimethylbenzylammonium[a] chloride, soon after Domagk's initial paper, and the extensive series of publications through the intervening years on this and related compounds exemplify the important role the quaternary ammonium salts play in the search for an ideal antiseptic.

The purpose of this monograph is to accumulate in the pages of one volume a review of the history, chemistry, biology, and application of the quaternary ammonium salts that have appeared in print to date.

2. Historical

Probably no other investigator or group of investigators has made a more thorough study of the chemistry and bacteriology of a single series of quaternary ammonium salts than the Jacobs group at the Rockefeller Institute. The synthesis and chemistry of approximately 200 hexamethylenetetraminium salts were described by them in the first group of eight publications.

While most of the current reviews on the quaternary ammonium antiseptics tend to indicate that the Jacobs papers were the original contributions on these salts, reference is made in one of their publications to earlier studies on these compounds as follows: "The preparation of quaternary salts by the addition of hexamethylenetetramine to halogen ketones has already been the subject of a study by Mannich and Hahn (1911)[1] who used these substances as the starting preparation of a few aminoketones according to the method of Delepine (1897)."[2] Reference may also be made at this point to the early publication of Reychler (1913)[3] who described the synthesis and colloidal properties of cetyltriethylammonium iodide.

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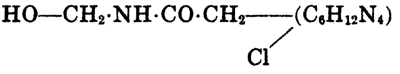
[a] See page 198 for trade names and distributors.

A review of the first series of papers by Jacobs and Heidelberger[4-11] failed to reveal any evidence that the hexamethylenetetramine salts had been tested for antiseptic or germicidal action. Nevertheless, in subsequent publications[12-14] by these authors and their coworkers, in which additional derivatives were synthesized on the hexamethylenetetramine nucleus, details of the bacteriology as well as the chemistry of these compounds were given. The authors again acknowledge an earlier publication relative to their investigations as follows: "Our attention was attracted first to this group of substances by the powerful bactericidal properties of the simplest representative, that obtained by the addition of hexamethylenetetramine to chloroacetylaminomethanol.



$$HO\text{—}CH_2\cdot NH\cdot CO\cdot CH_2\text{——}(C_6H_{12}N_4)\underset{Cl}{}$$

The compound was first prepared by Einhorn and Göttler (1908)[15] who also recognized its antiseptic properties." While this publication first suggests that a quaternary ammonium compound may have antibacterial properties, an article by Einhorn and his coworkers in 1905[16] makes mention of the pharmacological action and effects of hexamethylenetetramine upon fungi.

It is apparent, therefore, that contrary to the statement made by McCulloch, Hauge, and Migaki[17] that the discovery of the quaternary ammonium germicides originated from Jacobs and his coworkers, the latter authors themselves acknowledge that the priority of their description belongs to European investigators.

The extensive and classical studies on the relationship of chemical structure to antibacterial activities of the hexamethylenetetraminium salts, described in the Jacobs papers, apparently failed to arouse any interest in their use as antiseptics by other investigators. This may be inferred from the failure of the literature of the following years to reveal any evidence that the more active compounds were used as medicinals or general disinfectants. Almost 20 years elapsed before any further significant contributions were made to studies on germicidal quaternary ammonium compounds.

While Hartmann and Kagi[18,19] described the synthesis and referred to the antibacterial action of quaternary ammonium compounds in 1928 and 1929, beyond doubt the greatest contribution to the present-day use of surface active quaternary ammonium salts as germicides and antiseptics was made by Domagk[20,21] in 1935, who focused attention on the high antibacterial activity of alkyldimethylbenzylammonium chloride solutions. This is evident from 25 or more publications that appeared in foreign journals in the same and following year on the successful use of the compound in surgical procedures.

One of the earliest publications on the use of alkyldimethylbenzylammonium chlorides as antiseptics in this country was made by Dunn in 1936.[22] The number of papers that have been published on the latter and related groups of compounds since that date is evidence of the important part that the quaternary ammonium compounds play in the field of antisepsis.

Preparations of most of the quaternary ammonium compounds that have been described during the past several years consist mainly of variations of substituents attached to the nitrogen nucleus. These new derivatives include the following: (a) cyclic amines, pyridine, picoline, lutidine, etc., of the type cetylpyridinium chloride (Shelton *et al.*[23,24,25,26,27]); (b) compounds in which two long-chain alkyl radicals (C_4 to C_{16}) are introduced on the nitrogen atom to give a series of dialkylmethylbenzylammonium chlorides (Kuhn, Jerchel, and Westphal;[28] Kolloff, Wyss, Himelick, and Mantele[29]); (c) those compounds in which amide linkages are introduced in the long chain giving rise to compounds of the type *N*-(lauric acid ester of colaminoformylmethyl)pyridinium chloride (Epstein, Harris, and Katzmann[30,31,32]); (d) compounds containing an aromatic ring in the long chain of the type *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride monohydrate (Rawlins, Sweet, and Joslyn[33]); and (e) compounds containing unsaturated links in the long-chain alkyl groups of the type 9-octadecenyldimethylbenzylammonium chloride[b] (Valko and DuBois[34]). Some compounds, however, have been described in which the benzene ring has been substituted by chlorine or nitro groups. They will be discussed later.

The early application of the surface-active quaternary ammonium compounds (alkyldimethylbenzylammonium chlorides) was in surgical procedures. This included not only sterilizing or cleansing the operative field for surgery but scrubbing the surgeon's hands and arms to destroy organisms. With the evidence that was accumulated on the high germicidal action of the compounds against pathogenic, as well as nonpathogenic bacteria, consideration was given to their application as a general disinfectant. At the present time some of the surface-active germicides are being employed in many food industries such as fisheries, bakeries, cold storage warehouses, egg-breaking, dehydrating, dairy, and pasteurizing plants. Three of the compounds are listed in *New and Nonofficial Remedies,* 1949 and one (alkyldimethylbenzylammonium chloride) appears in the XIIIth edition of the *United States Pharmacopoeia* under the name Benzalkonium Chloride.

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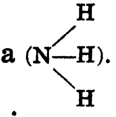
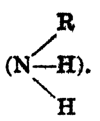
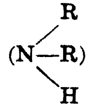
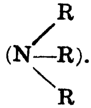
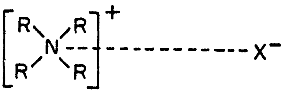
[b] See page 198 for trade names and distributors.

3. Definition

The surface-active quaternary ammonium compounds have frequently been designated in the literature as "cationic detergents," or "cationic wetting agents." In general, the quaternary ammonium germicides marketed today materially lower the surface tension of water, and thus are surface-active agents. Surface agents may have both a wetting action and a detergent action. These terms are not synonymous; it is entirely possible for a surface agent to have good wetting and poor detergent properties, or vice versa. Most of the present quaternary ammonium germicides have relatively good wetting action and relatively poor detergent action. Many investigators today object to the use of the term "cationic detergents" in describing the surface-active quaternary ammonium antiseptics. However, in order to justify references which have already been cited in the literature on these compounds, the expression will be used when reviewing these publications.

The term "quaternary ammonium" represents one of four possible classes of organic nitrogen compounds that may be derived from ammonia . The first three types are obtained by replacing the three primary valences on the *N* atom while the fourth type is concerned with its additional co-valent bond. By replacing one of the hydrogens on ammonia with an alkyl group, i.e., methyl (CH_3), ethyl (C_2H_5), dodecyl ($C_{12}H_{25}$), etc., a primary amine is obtained . Replacement of two hydrogens by alkyl groups results in a secondary amine  $\left(N\begin{array}{l}\diagup R\\ -R\\ \diagdown H\end{array}\right)$. A tertiary amine is formed by substituting three alkyl groups in the position held by the three hydrogens on the molecule  $\left(N\begin{array}{l}\diagup R\\ -R\\ \diagdown R\end{array}\right)$.

In the three classes of amines mentioned, the nitrogen has a valence of 3. However, a fourth amine is possible where the nitrogen has a co-valence of 5, or where five atoms may be considered as pertaining to the nitrogen nucleus. These compounds are then designated "quaternary ammonium" and are of the following general configuration:



where X represents a halogen, hydroxyl, or alkoxy radical. The latter are ions and satisfy the "5th valence." They are "bound" differently from the remainder of the atoms in the molecule in that they assume a negative electric charge.

Surface-active agents may be classified as (1) nonionic; or (2) ionic. The ionic class may be subdivided as (a) cationic, in which the lipophilic (oil attracting) group becomes the cation (+); (b) anionic, in which the lipophilic group becomes the anion (—). The surface-active quaternary ammonium compounds ionize in water as cationic compounds in which the long-chain part of the molecule is positively charged.

4. Chemistry

SYNTHESIS

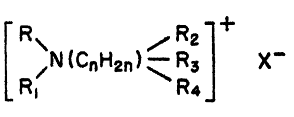
1. *The Quaternary Salts of Hexamethylenetetramine.* In the introductory paragraphs of the first of the series of the Jacobs and Heidelberger papers[3] on the quaternary hexamethylenetetraminium salts are given the principal reasons for the interest of the authors in synthesizing quaternary compounds on the hexamethylenetetramine nucleus. The latter had been used, since its introduction in medicine by Nicollaier in 1894, as an internal antiseptic. This action was later believed to be due to the liberation from it of formaldehyde.

Taking advantage of the chemical nature of hexamethylenetetramine, which functions as a tertiary base and reacts as such with certain types of organic halides to form quaternary ammonium salts, Jacobs and Heidelberger altered the molecule in various manners by the introduction of chemical groups to yield compounds with definite bactericidal power.

Wohl[85] had earlier prepared the methyl and ethyl derivatives of hexamethylenetetramine; and Delepine,[2] the allyl, amyl, and benzyl homologues. Mannich and Hahn[1] synthesized several aminoketones and glycinephenolic esters; and Einhorn and Göttler,[15] the halogenacetamide and oxymethyl derivatives of the compound. While several other investigators described the synthesis of additional interesting salts of hexamethylenetetramine, the latter authors were the first to call attention to the antiseptic action of the compounds.

The quaternary salts synthesized by Jacobs and Heidelberger included the following: (1) the substituted benzyl halides; (2) monohalogenacetylbenzylamines; (3) monohalogenacylated amines; (4) monohalogenacylated simple amines, ureas, and urethanes; (5) monohalogenacetyl derivatives of aminoalcohols; (6) halogenethyl esters and ethers; (7) ω-halogen derivatives of aliphatic-aromatic ketones; (8) and a group of miscellaneous compounds containing aliphatically bound halogens.

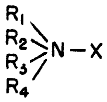
2. *The Quaternary Salts of Alkylammonium Halides.* Hartmann and Kagi[18] in 1929 described the synthesis and later[19] obtained one of the first United States patents on a series of quaternary ammonium salts. These compounds were prepared by the acylation of alkylenediamines and have the following general formula:



$$\left[{R \atop R_1}\!\!>\!N(C_nH_{2n})\!<\!{R_2 \atop {R_3 \atop R_4}}\right]^+ \quad X^-$$

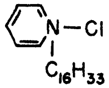
In which $R = H$, acyl or a hydrocarbon radical; $R_1 =$ acyl; R_2, R_3, and $R_4 =$ a hydrocarbon radical such as alkyl, aryl, or aralkyl; $X =$ a monovalent anion; and $n =$ a whole number. Reference is made in their first article to unpublished data of Doerr who found compounds in this series to have an antiseptic action. Their patent refers to the compounds as being merely for "technical and therapeutic purposes."

Domagk[20] described a large series of compounds in which at least one radical (R) is a long-chain aliphatic group (C_8H_{17} to $C_{18}H_{37}$) either plain or substituted. The other three radicals (R) may be either one, two, or three lower alkyl- or benzyl-bound or one aliphatic-bound phenyl radical in the following general formula:



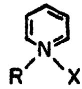
The radical —X is either a halide or another anion, i.e., sulfate or acetate. Compounds similar to the latter but with two C_8 to C_{16} alkyl groups instead of one long-chain alkyl radical in the (R) positions were described by Kuhn, Jerchel, and Westphal.[28] The observations of Domagk, therefore, proved that the bactericidal properties of quaternary ammonium compounds are not restricted to the quaternary salts of hexamethylenetetramine.

3. *The Quaternary Salts of Alkyl Pyridinium Halides.* Shelton, *et al.*[23-27] reported on a large series of quaternary ammonium salts derived from aliphatic or heterocyclic amines and observed that optimal bactericidal activity is present in compounds having a cetyl($C_{16}H_{33}$) radical. The remaining three radicals (R) are very simple, i.e., three methyl groups as in the case of cetyltrimethylammonium bromide, or they can be arranged in a cyclic configuration as in the case of cetylpyridinium chloride of the following structure.

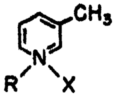


A series of dodecylamines were also described by Leffler and Volwiler[36] who compared the effects of various ring structures involving the nitrogen atom and their bactericidal activity.

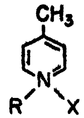
Kolloff, Wyss, Himelick, and Mantele[29] compared the germicidal activity of a series of quaternary ammonium derivatives which are alkyl pyridinium halides with the corresponding alpha- and gamma-picolinium compounds. The chemical relationship of these quaternary salts may be represented as follows:



Pyridinium alkyl halides



β-picolinium alkyl halides

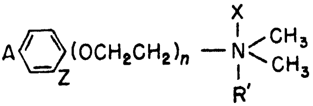


γ-picolinium alkyl halides

in which R = $C_{12}H_{25}$, $C_{14}H_{29}$, or $C_{16}H_{33}$, and X = halogen. The method used in the preparation of the compounds was essentially the procedure reported by Knight and Shaw.[37] Kolloff and his coworkers were interested in this series of quaternary ammonium salts, since previous reports by Coulthard, Marshall, and Pyman[38] indicated that the introduction of a methyl group into the phenol molecule increases the germicidal activity of the compound.

4. *The Quaternary Salts Containing an Ether Linkage.* Taub and Leuchs[39] described a series of quaternary ammonium compounds having bactericidal and fungicidal properties, in which at least one higher molecular aliphatic ether radical is present and separated from the quaternary nitrogen radical by at least two carbon atoms.

Rawlings, Sweet, and Joslyn[33] prepared and studied the germicidal activity of a series of quaternary ammonium salts in which the cation contained one long alkyl or alkylarylpoly-(oxyalkyl) chain, one short aralkyl group and two lower alkyl groups. The group of quaternary ammonium compounds were of the following general type:



in which A is alkyl, aryl, aralkyl, or cycloalkyl; Z is halogen, alkyl, aryl, or cycloalkyl; R′ is hydrogen, methyl, or substituted methyl; X is chloride or other anion, and *n* is 2 or 3. They concluded from their studies that the general configuration of the molecule of the quaternary ammonium salt is as important as the exact nature of its substituents. Furthermore, closed-ring substituents on the aromatic nucleus are definitely inferior to alkyl groups with regard to increasing germicidal activity. In the series of quaternary ammonium salts tested, Rawlins *et al.*, selected *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride as the most promising general germicide.

5. *The Quaternary Salts in Which Oxygen Is Introduced as Amide or Ester Linkages.* Epstein, Harris, and Katzmann[30,31,32] described a group of quaternary ammonium compounds with germicidal action in which the lipophile group is interrupted by carboxyl and amide linkages.[c] The derivatives are characterized by the cationic part of

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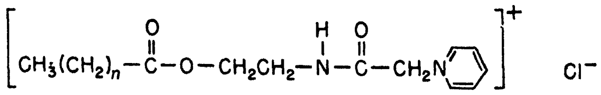
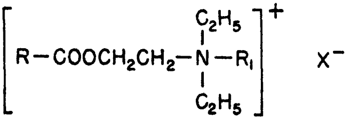
[c] The interests of Epstein, Harris, and Katzmann in the synthesis of quaternary ammonium germicides apparently originated from the earlier work of Harris (1933)[40,41,42] who synthesized and described the use of a large series of quaternary ammonium compounds as antispattering agents for margarine.

the molecule having an acyl group, a "colamine" group, and a formyl-methyl quaternary ammonium radical. The lauric acid ester of colaminoformylmethylpyridinium chloride was selected as the most active germicide with a corresponding low toxic action to tissues. The introduction of the carboxyl and amide groups into the molecule has an effect upon the functional properties of the compound. A study was made of the effect on the bactericidal value of these compounds in relation to the chain length of the fatty acid radical which enters in the lipophile group and their respective relations to detergency (C_8 to C_{18}). The general structural formula of a compound representing this group may be given as follows:



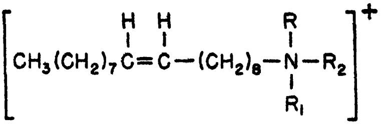
Woodruff, Aspergren, and Mantele[43] prepared and studied the antibacterial effects of quaternary ammonium salts with ester groups and found that the presence of the latter in the molecule had no appreciable effect on the germicidal value. Their compounds were characterized by the following general configuration:



$$\left[R-COOCH_2CH_2-\overset{\displaystyle C_2H_5}{\underset{\displaystyle C_2H_5}{\overset{|}{\underset{|}{N}}}}-R_1 \right]^+ X^-$$

in which R = C_8 to C_{17}; R_1 = CH_3, or $CH_2CH = CH_2$; and X = halide.

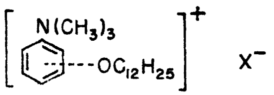
6. *The Quaternary Salts Containing Unsaturated Links in the Long-Chain Alkyl Group.* Valko and DuBois[34] synthesized and tested a large number of quaternary ammonium salts in which the long-chain alkyl group is interrupted by an unsaturated link. The 9-octadecenyl compounds of this series of germicides have the following general structure:



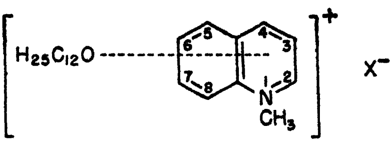
$$\left[CH_3(CH_2)_7\overset{\displaystyle H}{\overset{|}{C}}=\overset{\displaystyle H}{\overset{|}{C}}-(CH_2)_8-\overset{\displaystyle R}{\underset{\displaystyle R_1}{\overset{|}{\underset{|}{N}}}}-R_2 \right]^+ X^-$$

where R and R_1 = hydrogen, methyl, amyl radicals; R_2 = hydrogen, methyl, ethyl, propyl, amyl, dodecyl, hydroxyethyl, allyl, ethoxyethyl, benzylphenethyl, and -phenyl propyl groups; and X = chloride or bromide. Maximum germicidal activity against *Staphylococcus aureus* was obtained with the derivatives containing dimethylethyl, dimethylpropyl, and dimethylbenzyl constituents on the nitrogen atom.

7. *The Quaternary Salts Containing Substituents in the Aromatic Nucleus.* In contrast to the afore-described series of compounds in which the substituents on the quaternary *N* atom were varied, several series of compounds have resulted by substitution on the benzene ring. Kuhn and Jerchel[44] described a series of quaternary salts of aminophenolethers. These compounds are of particular interest in that unlike many of the other quaternary ammonium salts that contain the long-chain aliphatic groups attached directly to the *N* nucleus, the alkyl group in the aminophenolether series is separated from the *N* atom by a benzene ring. In bacteriological studies on the methosulfates of *o*-, *m*-, and *p*-trimethylammoniumphenol-*n*-dodecylether, the compounds proved to be as active as, and in many instances more active than, dodecyldimethylbenzylammonium bromide and loroldimethylbenzylammonium[d] chloride. The general chemical structure of the aminophenol ethers is as follows:



Kuhn and Westphal[45] synthesized and tested a group of oxyquinoline ethers which were also characterized by having the long-chain aliphatic groups separated from the *N* nucleus by an aromatic group. They noted that the position of the lauryloxy group in the molecule had no influence on the germicidal action of quinoline derivatives. The methosulfate salts of 3-, 6- and 8-oxyquinolinedodecylether have the following general structure:



Kolloff, Wyss, Himelick, and Mantele[29] described the germicidal action of several alkyl pyridinium and picolinium quaternary ammonium salts that have a methyl radical in the aromatic nucleus. Since the presence of the methyl group in the position mentioned did not significantly increase the activity of the compounds, they were not considered of further investigative interest.

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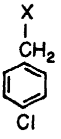
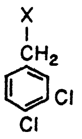
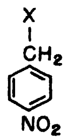
[d] *Lorol* represents the homologous series of straight-chain alkyl groups from C_8H_{17} to $C_{18}H_{37}$. The percentages of the alkyl radicals in the compound are in the same ratio as they occur as fatty acids in coconut oil.

Compounds with substituents on the aromatic nucleus were also described by Leuchs.[46] These substituents consist of at least one alkyl, alkoxy, halogen, or nitro group, and are arranged on the molecule in the following manner:



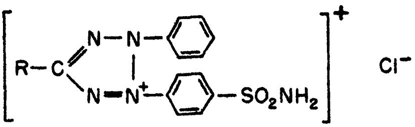




in which X = *N* nucleus with variations in substituents.

DuBois and Dibblee[47] [48] more recently described the antibacterial effects of an alkyldimethylbenzylammonium chloride which contains chlorine substituents in the 3 and 4 positions on the benzene nucleus. A group of alkyldimethylbenzylammonium chlorides has also been studied by Lawrence, Kwartler, Wilson, and Kivela[49] in which substitutions were made on the aromatic or benzyl portion of the molecule. The compounds include 2-chlorobenzyl-, 4-chlorobenzyl-, 2,4-dichlorobenzyl, 3,4-dichlorobenzyl-, isomeric dichlorobenzyl-, isomeric trichlorobenzyl-, and 4-nitrobenzyl derivatives of alkyldimethylbenzylammonium chloride with alkyl radicals ranging from C_8 to C_{18}. An evaluation of the relative germicidal actions of these quaternary ammonium salts will be the subject of a subsequent section in this review.

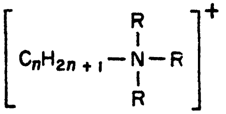
8. *The Quaternary Salts of Sulfonamides.* Jerchel[50] described the synthesis and antibacterial effects of a group of quaternary ammonium salts of tetrazole on which were introduced a sulfonamide group. The general structure of this series of compounds is of the following:



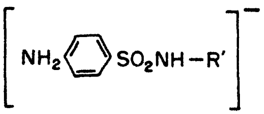
where R= CH_3, C_3H_7, C_6H_{13}, C_7H_{15}, $C_{11}H_{23}$. 2-phenyl-3-*p*-sulfamidophenyl-5-undecyltetrazolium chloride, in the series, was found to be as active as diphenylundecyltetrazolium chloride and loroldimethylbenzylammonium chloride in inhibiting the growth of *Streptobacterium plantarium.* However, the quaternary ammonium sulfonamide was considerably less active than the unsubstituted diphenylundecyltetrazolium compound against *S. aureus.*

The desirability of having available a single compound which exhibits not only sulfonamide action but also bactericidal, surface-acting properties has been suggested by Barry and Puetzer.[51] These workers implied that a compound containing a highly germicidal quaternary ammonium residue as the surface-active portion of the molecule, combined with a sulfonamide group, may be useful in nasal therapy and for other chemotherapeutic applications. The chemistry of several of these compounds, namely the cetyltrimethylammonium, cetylpyridinium, and laurylpyridinium salts of one or more of the commonly known sulfonamides, was described by the workers mentioned. They concluded from their studies that the compounds were highly bactericidal against *S. aureus* and *Escherichia coli, in vitro*—in some instances the compounds were more effective than the corresponding ammonium components contained in the molecule.

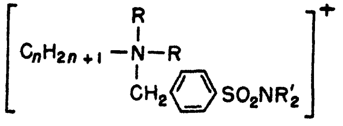
Studies by Lawrence and Goetchius[52] have indicated that several other quaternary ammonium sulfonamides are likewise effective in high dilutions in destroying a variety of pathogenic bacteria. Unlike the compounds described by Barry and Puetzer, in which the sulfonamide portion of the molecule is the anion, these studies involved the use of compounds in which the sulfonamide radical is part of the cation. The difference in the structural configuration of the two types of quaternary ammonium sulfonamides is as follows:



Cation
Where R = CH_3 or R_3N = pyridinium



Anion
Where R′ = thiazole, diazine, pyridine, or methylpyridine



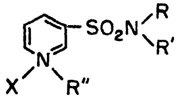
Cation
Where R = CH_3, pyridine or quinoline; where R′ = H or alkyl groups.

$[\mathrm{Br}]^-$

Anion

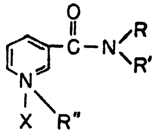
Tests on the relative germicidal actions of several of these quaternary ammonium sulfonamides revealed that 4-sulfanilamidobenzyltetradecyldimethylammonium bromide and 4-dodecylsulfanilamidopyridinium bromide gave phenol coefficient values of 233 and 267 against *S. aureus* and *E. typhosa* respectively. The antibacterial action of these and the other derivatives were also studied against a wide variety of Gram-negative and Gram-positive bacteria in the presence of broth.

Zienty[53] described the methods of synthesis and germicidal activity of 18 quaternary ammonium salts of *N′*-substituted pyridine-3-sulfonamides. The general structural configuration of the latter compound is as follows:



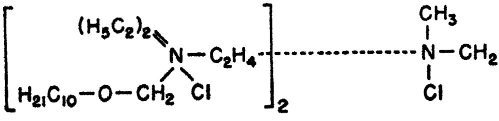
where R and R′ = alkyl radicals H, C_2H_5, C_8H_{17} to $C_{16}H_{33}$; R″ = CH_3, C_7H_7, C_3H_5, $C_{12}H_{25}$, or $C_{16}H_{33}$; X = Cl, Br, I or SO_4CH_3. In determining the germicidal activity of 18 of these quaternary ammonium salts, several were found to compare favorably with the phenol coefficient values reported for alkyldimethylbenzylammonium chloride.

The synthesis and germicidal activity of 27 salts of nicotinamide, *N′*-*N′*-diethyl nicotinamide and 4-(nicotinyl) morpholine were also described by Zienty.[54] The general structure of this group of compounds is as follows:

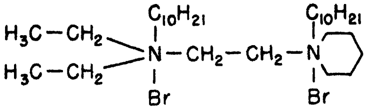


Phenol coefficient determinations on this group of quaternary salts of pyridine-3-carboximides revealed them to have some degree of germicidal activity against *S. aureus*. However, they were less active than the corresponding pyridinium or picolinium compounds.

9. *Miscellaneous Quaternary Salts.* Brief mention may be made of a series of polyammonium compounds described by Taub and Leuchs[55] which were found to have bactericidal and fungicidal properties. The complexity of structure and the possible quaternary ammonium compounds that can be synthesized is evidenced by the following formulas given in the Taub and Leuchs patent for two of the polyammonium salts:



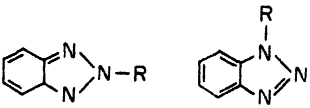
bis-(diethyldecyloxymethyl-chloroammonium-ethyl) methyl-decyloxymethylammonium chloride.



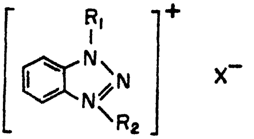
N-diethyldecylbromammoniumethyldecylpiperidinium bromide

Reference may also be made to quaternary ammonium compounds which have been prepared by Taub,[56,57] Hahl,[58] Leuchs,[59] and others, in which a sulfur atom has been introduced as an ether linkage in the high molecular aliphatic chain. In studying the antibacterial effects of a series of sulfonium iodides, Kuhn and Dann[60] pointed out that the laurylbenzylmethylsulfonium salt is considerably less active than the corresponding lauryl derivative of benzyldimethylammonium bromide. The correlation between structure and bactericidal activity of unusual quaternary ammonium compounds was also studied by Eldred and Niederl.[61] These investigators found that factors such as the general molecular structure, type of *N*-alkyl radicals and length of carbon chain, influence the bactericidal activity of quaternary ammonium salts.

Kuhn and Westphal[62] described an interesting group of quaternary salts that were obtained by reacting benzotriazole-alkali salts with halogen-alkyl and dialkyl-sulfate to form alkyl-benzotriazole of the following configurations:

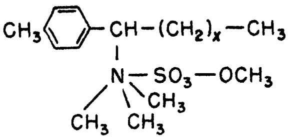


This reaction can proceed further by certain methods to give 1,3-dialkylbenzotriazolium salts as the following:



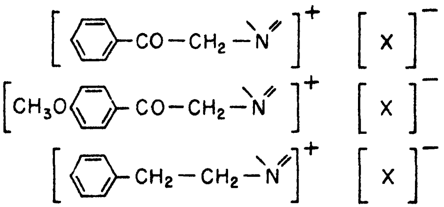
A compound of this type in which $R_1 = C_{12}H_{25}$, $R_2 = C_2H_5$, and $X = Br$ was reported to be markedly more active than the corresponding lauryl dimethylbenzylammonium bromide in killing and inhibiting staphylococci, *E. coli* and *S. schottmülleri.* In a report by Rawlins, Sweet, and Joslyn[38] some time later, the same benzotriazolium compound did not prove to be as active against microorganisms as indicated by the foreign investigators.

Grumbach[63] described a compound which is a mixture of methosulfate quaternary ammonium bases of the following structure:



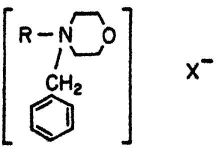
where $x = 6$ to 16. Comparable inhibiting and killing actions were obtained with this compound and alkyldimethylbenzylammonium chloride.

Hartwell and Kornberg[64] gave the methods of synthesis of a number of new quaternary ammonium salts that were prepared for a study on the chemotherapy of cancer. These compounds are of the general structural configuration:



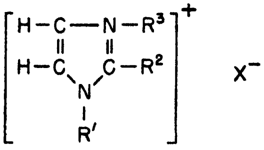
where X = a halide. No mention was made by Hartwell and Kornberg concerning the action of these salts against tumor tissues or bacteria. There was, furthermore, no mention made of the pharmacology, toxicology, or other properties of these derivatives on animal tissues.

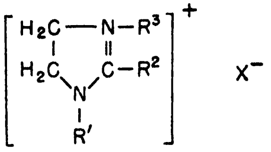
Niederl, Niederl, and McGreal[65] were issued a patent for a group of *N*-benzyl-*N*-alkylmorpholinium halides of which several were reported to have phenol coefficient values as high as 500. As an example the latter figure was obtained as the maximum in the series on the *N*-myristyl or C_{14} compound. The *N*-cetyl (C_{16}) homologue on the other hand, gave a phenol coefficient value of but 50. The general configuration of this class of compounds is as follows:



where R = alkyl group, and X = a halide.

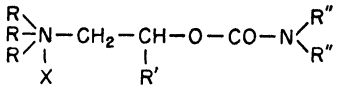
Shepard and Shonle[66] prepared and tested a group of 54 imidazolium and imidazolinium salts of the general structures given as follows:





where R = alkyl or aralkyl, and X = anion. Maximum activity was found in those compounds having C_{12} or C_{14} substituents in the 1 or 2 position. Brief mention may also be made here to the parallelism that existed between the bacteriostatic effect of the compounds and their ability to hemolyze washed red blood cells.

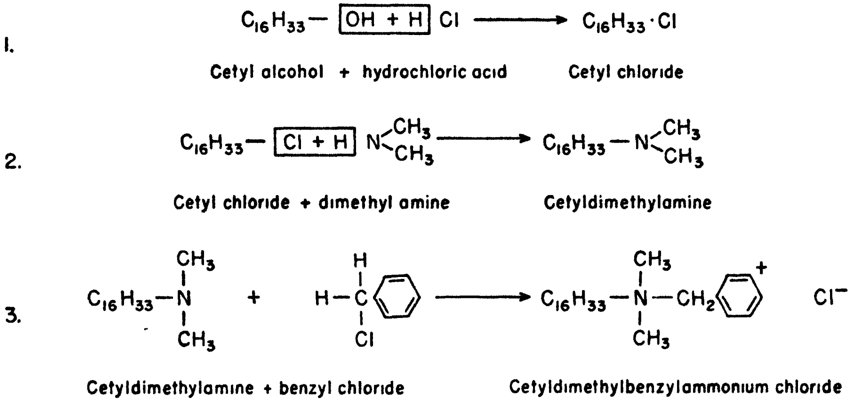
A patent was issued to Swan and White[67] on a series of "choline-like" compounds which can also be considered quaternary ammonium salts with the general structure as follows:



where R = lower alkyl radicals; X = anion; R′ = H and lower alkyl radicals; R″ = alkyl radicals having more than three carbon atoms, and aryl radicals. These compounds exhibit valuable pharmacological properties in that they were shown to have mydriatic and cycloplegic actions. The authors report that the products are similar in action to atropine and homatropine. Of particular interest to this review is the claim made that these drugs also have antiseptic properties.

By concluding this section of the review on the variety or types of quaternary ammonium salts that can be synthesized and have been described in the literature, it is not inferred that the list is a complete one. Reference may be made to an excellent review on quaternary ammonium compounds by Wakelin[68] who presents a more detailed account on the development of this class of germicides.

Most of the important surface-active quaternary ammonium compounds have their primary source in the natural vegetable or animal oils or resins (palm oil, coconut oil, colophony, etc.). These oils are the condensation products of glycerin and long-chain fatty acids. It is possible to break down the oils by hydrolysis with steam, by saponification with alkalies or by catalytic hydrogenation. Catalytic hydrogenation will give glycerol and the long-chain alcohol corresponding to the groups present in the fatty acid or acids. The quaternary ammonium type of compounds described by Domagk is made by reaction of the long-chain alcohols obtained from the fatty acids of coconut oil with hydrochloric acid, treatment with dimethylamine, and, finally, reaction with benzyl chloride. An illustration of this series of reactions may be given as follows:


1.
C16H33— OH + H Cl → C16H33·Cl
Cetyl alcohol + hydrochloric acid
Cetyl chloride
2.
C16H33— Cl + H N(CH3)2 → C16H33—N(CH3)2
Cetyl chloride + dimethyl amine
Cetyldimethylamine
3.
Cetyldimethylamine + benzyl chloride
Cetyldimethylbenzylammonium chloride

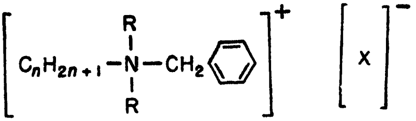
Quaternary ammonium compounds may be prepared from a mixture of alkyldimethylammonium chlorides. When coconut oil is subjected to catalytic hydrogenation the mixed glycerides of the acids in the oil are broken down to a mixture of fatty alcohols. The ratio of the individual alcohols obtained in the reaction is the same as in the naturally occurring fatty acids in coconut oil. Further treatment of the alcohols results in the corresponding chlorides. The mixed chlorides are known as "lorol chloride" (see footnote d, p. 10) which distinguishes them from $C_{12}H_{25}\cdot Cl$, the latter being known as "lauryl chloride." The distribution of the fatty acids in coconut oil, which is the primary source of some of the mixtures of alkyl groups in certain commonly known surface-active quaternary ammonium compounds, is as follows (Longenecker, 1939[69]):

Acid	*Wt. per cent*	*Mole per cent*
$C_5H_{11}COOH$—Caproic acid	0.8	1.4
$C_7H_{15}COOH$—Caprylic acid	5.4	7.8
$C_9H_{19}COOH$—Capric acid	8.4	10.2
$C_{11}H_{23}COOH$—Lauric acid	45.4	47.2
$C_{13}H_{27}COOH$—Myristic acid	18.0	16.4
$C_{15}H_{31}COOH$—Palmitic acid	10.5	8.5
$C_{17}H_{35}COOH$—Stearic acid	2.3	1.7
$C_{19}H_{39}COOH$—Arachidic acid	0.4	0.2
$C_{15}H_{29}COOH$—Hexadeconic acid	1.3	1.1
$C_{17}H_{33}COOH$—Oleic acid	7.5	5.5
$C_{17}H_{31}COOH$—Linoleic acid	Trace	Trace

The high percentage of lauryl and myristyl components in coconut oil makes the latter one of the most suitable primary sources for preparation of some of the mixed alkyl quaternary ammonium compounds. Evidence will be presented later to show that the most active germicides in the cationic series are those containing all, or a predominance of lauryl, myristyl or cetyl (C_{12}, C_{14}, C_{16}) alkyl groups in the molecule.

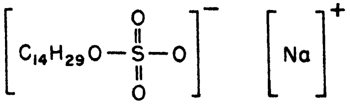
IONIZATION

The surface-active quaternary ammonium compounds ionize in water as cationic compounds in which the long-chain part of the molecule is positively charged and may be illustrated as follows:



Cation (lipophil) Anion (hydrophil)

The anionic surface-active agents, on the other hand, ionize in water with the long-chain alkyl group in the anion while the metal (sodium, potassium, etc.) fraction of the molecule becomes the cation. An example of the structural configuration of an anionic compound is sodium tetradecyl sulfate:



$$\left[C_{14}H_{29}O-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}-O\right]^{-}\quad\left[Na\right]^{+}$$

Anion (lipophil) Cation (hydrophil)

The nonionic surface-active agents are generally esters of fatty acids, formed by combining glycerin, or other polyhydroxyalcohols, with one fatty acid radical, leaving a sufficient number of hydroxyl groups to balance the lipophilic nature of the long chain. This results in two nonionizable groups which give very useful wetting agents. An example of the nonionic class is glycol monostearate:

$$[C_{17}H_{35}CO{-}O{-}CH_2CH_2OH]$$

Lipophil Hydrophil

The relationship between the ionic charges upon the surface-active ions and the bactericidal effects demonstrated by surface-active agents will be the subject of a later section of this review. Suffice it to say here that Ralston[70] has postulated that surface-active ions have a tendency to associate and form ionic micelles. These micelles, the highest charged particles present in aqueous systems of all colloidal electrolytes, instead of simple ions are believed to be responsible for the destruction of bacteria.

Valko,[71] however, has shown that the killing concentrations of a surface-active compound for bacteria, in the absence of added electrolytes is considerably higher than in the presence of electrolytes, yet definitely lower than the critical concentration for optimal micelle formation. While some micelles may be present below the critical concentration, the forces acting between the surface-active ions and proteins is greater than the forces acting in the micelles between the surface-active ions themselves. The micelles, therefore, may more readily dissociate into single ions when reacting with the protein substrates of bacterial cells.

An extensive discussion and bibliography has been prepared by Ralston and his co-workers[70,72,73,74] on the structure and properties of colloidal electrolytes, including quaternaries, dealing with the micelle theory devised by McBain,[75] which explains the anomalous behavior of electrolytes in water. Adam and Shute[76] have noted certain anomalies in the surface tension of quaternary ammonium compounds which they attribute, in part, to micelle formation in certain concentrations of the compounds in water.

Stuart[77] has more recently explained variations in germicidal activity of quaternary ammonium solutions as due to differences in the way in which they hydrolyze in water. This apparently results from some salts being derived from weak bases and strong acids and others from strong bases and weak acids. He noted, moreover, that the pH of a solution of a quaternary ammonium solution may change between the time it is originally prepared in water and the time it is tested several hours later. This change in pH has a definite influence on the germicidal action. For example, when tested at pH 5.47, a quaternary ammonium solution was found to kill a test organism in a dilution of 1:10,000, whereas, at pH 7.03, the same solution destroyed the organisms in a 1:18,000 concentration.

ACTION ON METALS

Hornung[78] early recommended the use of an antirust agent ("soda") for the prevention of oxidation of metals treated with a quaternary ammonium germicide. Addition of the alkali did not affect the antibacterial activity of the solution. While the present-day manufacturers of quaternary ammonium salts claim that their products are no more corrosive to metals than water, they do, nevertheless, advocate the use of an antirust compound in solutions for the sterilization of surgical instruments, metal dairy utensils, etc. This is particularly the case where storage of the metal objects in the germicide more than momentarily is a routine procedure.

An effective and commonly used antirust agent for quaternary ammonium germicides is sodium nitrite.[79,80,81,82,83] Sodium carbonate and sodium borate are also used, but prevention or delay in oxidation of metals is not as pronounced when these compounds are employed. A combination of sodium nitrite and sodium carbonate is considerably more effective than sodium nitrite alone. These compounds added to quaternary ammonium solutions do not reduce the bactericidal activity of the germicide. In fact, there are indications that solutions containing the sodium salts are more germicidal than those from which the salts are omitted. This is attributed to the increase in pH.

The amount of antirust agent necessary to be added to quaternary ammonium solutions for the prevention of corrosion of metals is mainly dependent upon the concentration of the germicide that is being used. With but few exceptions, the quaternary ammonium germicides available on the market today are supplied without an antirust agent. Furthermore, since the concentrations available range from 100% to 0.1% in water, or are in the form of alcohol-acetone tinctures, one can readily see the difficulty that may be encountered in supplying a product containing an antirust agent if the product has to be diluted with water before actual use.

A series of studies were carried out in the writer's laboratories to determine the extent of corrosion that will occur on various metals treated with a range of dilutions of a quaternary ammonium germicide. Rubber was included in the test since in certain applications of the germicides, rubber connections, tubing, surgical gloves, etc., are sterilized by quaternaries. In this particular test, concentrations far exceeding those actually encountered under practical conditions of use were employed (10%, 20%, and 40%).

Samples of small strips of several metals were first highly polished on a carborundum wheel and, after thorough cleaning with trisodium phosphate in water, were placed in several concentrations of quaternary ammonium solutions. A control series consisting of tap water was included in the study.

Table 1 presents the corrosive action of the germicide and water upon the various metals, alloys, and rubber after two months' exposure. Three readings were made on each test sample: (1) the condition of the metal submersed in the solution; (2) the appearance of the metal at the liquid-air interface; and (3) the condition of the part of the metal extending out from the surface of the liquid or, in other words, in the air immediately above the solution.

From the data given in the table, it will be noted that iron is corroded more by water than by the germicidal solutions. Tool steel, on the other hand, is more readily corroded by the three concentrations of quaternary ammonium solution than by water. While the 10% solution of the germicide is no more corrosive than water to brass, the two higher concentrations of the test solution showed a greater action against this alloy. Stainless steel and Monel were the only test strips that completely resisted the rusting action of the germicidal solutions. Copper, aluminum, and lead were affected somewhat more by the germicide than by water, while tin was corroded to a greater extent by water than by the quaternary ammonium solution.

TABLE 1

CORROSIVE EFFECT OF QUATERNARY AMMONIUM CONCENTRATES ON RUBBER, VARIOUS METALS AND ALLOYS AFTER TWO MONTHS' EXPOSURE

SUBSTANCE	WATER (CONTROL)*			QUATERNARY SOLUTION†								
				10%			20%			40%		
	Liq.	Vap.	Air	Liq.	Vap.	Air	Liq.	Vap.	Air	Liq.	Vap.	Air
Rubber (synthetic)	0	0	0	0‡	0	0	0	0	0	0	0	0
Wrought iron	4	4	4	2	3	1	1	0	0	0	3	1
Tool steel	1	0	0	2	4	3	2	4	3	3	2	4
Brass	2	1	1	2	1	0	0	3	1	2	3	3
Stainless steel	1	0	0	0	0	0	0	0	0	0	0	0
Copper	0	0	1	4	4	1	4	4	3	4	4	1
Aluminum	1	2	0	1	3	2	2	3	3	2	3	2
Lead	2	2	2	1	3	3	1	3	3	1	3	3
Tin	4	4	1	0	2	2	1	2	0	4	4	2
Monel metal	0	0	0	0	0	0	0	0	0	0	0	0

*Figures 0 to 4 under "Water" columns indicate none to marked degree of corrosion with respect to clean metal.

†Figures 0 to 4 under "Quaternary Solution" columns indicate none to marked corrosion with respect to action of water on same metals.

‡Solutions in which rubber was tested were colored dark-brown to green. However, there was no evidence of a change in elasticity of the tubing.

In general, therefore, and with the exception of stainless steel and Monel metal, the quaternary ammonium solution in concentrations of 10% or greater is corrosive to metals. While a definite discoloration of synthetic rubber occurs when treated with the high concentrations of the compound, no change in the elasticity or other physical properties of the material could be noted. Although not indicated in the table, additional tests were made using the same metals exposed to 0.1% or less of the quaternary ammonium compound in water. The results confirmed the claims made by the manufacturer that, in the dilutions recommended for use, the germicide is no more corrosive than water.

In another series of tests a quaternary ammonium compound solution was treated with antirust agents and then added to wire nails. The latter were selected because they appeared to be more readily affected by the germicide than other metals or alloys. In this study, sodium nitrite, sodium carbonate, and sodium borate were used as the antirust agents. The alkaline salts were used alone and in combinations of sodium nitrite-sodium carbonate and sodium nitrite-sodium borate. The condition of the submersed nails, in closed containers, was followed for three weeks, and the data tabulated. pH readings were made on the solutions and the effects upon the metal scored with respect to visible rusting, formation of a sediment at the bottom of the container, and presence or absence of discoloration in the solution. The effects of diluting a concentrated antirust-germicidal solution showing little or no corrosive action was also investigated. The results of these studies are presented in Table 2.

Examination of the data given reveals the following pertinent points: Sodium nitrite in a concentration of 2.0% was effective in completely preventing the corrosion of wire nails by a 10% aqueous solution of the quaternary ammonium solution for a period of 21 days or more. Furthermore, the same solution could be diluted with 400 parts of water (1:4,000) and still show a favorable antirust action of the sodium salt. Sodium carbonate in concentrations of 1.5% to 2% resulted in definite anticorrosive properties of 10% of the quaternary ammonium solution. However, this action was not maintained upon dilution of the concentrates with water. Sodium borate (Borax) in a concentration as high as 2.5%, in 10% quaternary ammonium solution, gave but a suggestion of an anticorrosive action. It is evident from these findings that sodium nitrite alone, or a combination of sodium nitrite and sodium carbonate were the most effective salts used in the present investigation as antirust agents for quaternary ammonium solutions. No doubt there are other compounds that are equally if not more effective for this purpose than sodium nitrite. Nevertheless, availability, minimal cost and relatively nontoxic action are factors which favor the use of the latter salt as an anticorrosive agent for quaternary ammonium solutions.

Mueller, Bennett, and Fuller[84] compared the corrosive effects of quaternary ammonium compounds, phosphonium compounds, and a chlorine product on metals. Their studies revealed that none of the agents caused any appreciable corrosive action on stainless steel or Monel metal. Both the quaternary ammonium and phosphonium compounds were less corrosive than chlorine to tin. On the basis of their observations, Mueller and his co-workers found the surface-active agents to be satisfactory for dairy use in the sanitizing of equipment from the standpoint of corrosion.

TABLE 2

CORROSIVE ACTION OF ALKYLDIMETHYLBENZYLAMMONIUM CHLORIDE

Flask No.*	Gms. % $NaNO_2$	Gms. % Na_2CO_3	Gms. % Borax	pH	Time (Days)	Corrosion‡	Discoloration
1	...	...	...	7.1	21	++++	Marked
2	0.5	...	...		21	+++	Present
3	1.0	...	...	8.4	21	++	Slight
4	1.5	...	...		21	Trace	Slight
5	2.0	...	...		21	None	Slight
6	2.5	...	...	7.6	21	None	Slight
7	...	0.5	...		14	++	None
8	...	1.0			14	++	None
9	...	1.5	...		14	+	None
10	...	2.0	...	10.7	14	+	None
11	...	...	0.5	9.1	21	+++	Trace
12	...	...	1.0		21	++	None
13	...	...	2.5	9.3	21	++	None
14	1.0	0.5	...	10.4	21	+	Slight
15	1.0	1.0	...		21	+	Slight
16	1.0	1.5	...		21	Trace	Slight
17	1.0	2.0	...	10.9	21	Trace	Slight
18	1.0	...	0.5	9.1	21	+	Slight
19	1.0	...	1.0		21	+	Slight
20	1.0	...	2.5	9.3	21	+	Slight
As above except diluted to give 1:4000 alkyldimethylbenzylammonium chloride							
21	0.5	...	...		14	+++	Present
22	2.0	...	...		14	Trace	
23	...	0.5	...		14	++++	Marked
24	...	2.0	...		14	+++	Marked
25	1.0	0.5			14	+++	Slight
26	1.0	2.0		10.2	14	++	Slight

*All flasks contained 50 ml. of 10% aqueous quaternary ammonium compound to which *nails* and indicated amounts of chemicals were added.

‡ + to ++++ = Slight to marked corrosion.

In studies on the germicidal activity of *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride, Joslyn, Yaw, and Rawlins[85] briefly mentioned that this quaternary ammonium compound would not attack rubber, plastics, fabrics, and metals with the exception of copper and brass. It would appear advisable, nevertheless, for manufacturers of quaternary ammonium germicides to recommend the addition of an antirust agent to the sterilizing solution, particularly in those instances wherein surgical or dental instruments are allowed to remain in contact with the liquid for periods of one-half hour or more.[63,86]

COMPATIBILITIES AND INCOMPATIBILITIES

Domagk,[20] who first called attention to the high antiseptic properties of the quaternary ammonium germicides, was also first to indicate the incompatibility of an anionic detergent (soap) upon the cationic compounds. The incompatibility of soaps and quaternary ammonium germicides was also early reported by Hornung,[78] Schneider,[87] Hettche,[88] Neufeld, Schiemann, and Kuhn,[89] and Grumbach.[63]

Baker, Harrison, and Miller[79] noted the inhibiting action of the anionic compounds decyl sulfate and sodium taurocholate upon alkyldimethylbenzylammonium chloride. The cationic germicides have subsequently been found to be inactivated by many other types of anionic compounds (Valko and DuBois,[90] and Rahn[91]). Of particular interest were the observations of Valko and DuBois who noted that bacteria which are apparently "killed" by a cationic quaternary ammonium germicide may be revived by the addition of a high molecular anion, provided that application of the latter occurs within a time period of 10 to 30 minutes. Lawrence[92,93] has demonstrated, however, that while many of the anionic detergents will react with the quaternary ammonium compounds, they nevertheless do not meet the requirements for an ideal inactivator in determining true bacteriostasis or bactericidal titers of the cationic germicides. The anionics are in themselves active against microorganisms or fail to completely neutralize the antibacterial action of the cationic germicides. Similar observations on the inability of anionic compounds to completely neutralize cationic germicides have been made by Mueller, Seeley, and Larkin,[94] and Klein and Kardon.[95]

In the compounding of pharmaceutical preparations in which quaternary ammonium germicides are included as preservatives or bactericidal agents, certain compatibilities as well as incompatibilities have been noted. The latter may be evidenced by a physical change in the appearance of the solutions or mixtures in which a precipitate results on combining the quaternary ammonium solution with other substances. On the other hand, no change in the physical appearance of the combination may be noted, yet upon examining the material bacteriologically one can demonstrate little, if any, antibacterial activity in the preparation. In Table 3 are listed a number of compatibilities and incompatibilities that have been noted in combining alkyldimethylbenzylammonium chloride with various chemicals or compounds. The list of incompatibilities include solutions which form a precipitate upon the addition of the quaternary

TABLE 3

COMPATIBILITIES AND INCOMPATIBILITIES OF VARIOUS AGENTS WITH ALKYLDIMETHYLBENZYLAMMONIUM CHLORIDE

COMPATIBILITIES

1. Acetone
2. Acriflavine
3. Alcohol
4. Alkylated aryl poly ether alcohol
5. Amaranth
6. Benzocaine and sulfathiazole
7. Betain base 1%
8. Boric acid 0.5%
9. Camphor water
10. Chlorbutanol
11. Cinnamic aldehyde
12. Cocaine
13. Cresol
14. Ephedrine • HCl
15. Eosin Y
16. Formaldehyde
17. Fuchsin
18. Glycerin
19. Hydrazine hydrate 0.5% and 0.1%
20. Insulin
21. Lime water U.S.P.
22. Menthol
23. Oil of anise
24. Oil of cinnamon
25. Oil of clove
26. Oil of sassafras
27. Oil of spearmint
28. Penicillin
29. Polyethylene glycol
30. Pontocaine • HCl
31. Procaine • HCl
32. Rose water
33. Salicylic acid
34. Sodium borate
35. Sodium carbonate
36. Sodium nitrite
37. Sodium phosphate
38. Streptomycin
39. Tetrasodium pyrophosphate
40. Trisodium phosphate
41. Urea
42. Urethane

INCOMPATIBILITIES

1. Alkyl aryl sulfonate
2. Argyrol
3. Benzyl ephedrine
4. Boric acid 5%
5. Capsicum
6. Caramel
7. Citric acid
8. Hydrogen peroxide
9. Iodine
10. Methyl ester of *p*-hydroxybenzoic acid
11. Oil of peppermint
12. Phenylsalicylate
13. Pilocarpine nitrate
14. Pine oil
15. Potassium iodide
16. Potassium permanganate
17. Saccharin
18. Saponin
19. Silicates
20. Silver nitrate
21. Sodium citrate
22. Sodium hexametaphosphate
23. Sodium lauryl sulfate
24. Sodium tartrate
25. Sulfonated mineral oil
26. Tartaric acid
27. Yellow oxide of mercury
28. Zinc peroxide
29. Zinc sulfate

to them, or in which the antibacterial activity of the quaternary is partially or completely destroyed.

A list of compatibilities and incompatibilities of an alcoholic-acetone solution, as well as aqueous solutions of cetylpyridinium chloride, has also been prepared by Huyck.[96,97] However, he does not indicate whether or not the observations included bacteriological examination of the combinations. In studying the reaction between long-chain cationic salts and sodium hexametaphosphate, Powney[98] observed that a precipitate formed upon the addition of aqueous solutions of the phosphate to some quaternary ammonium germicides.

Huyck and Davy[99] attributed the incompatibility of soluble nitrates and alkyldimethylbenzylammonium chloride to the formation of a mixture of high molecular alkyldimethylbenzylammonium nitrates which are only slightly soluble in water. The incompatibility of soluble nitrates and aqueous solutions of the germicide depends upon the concentrations and character of the nitrates involved. They noted, furthermore, that when the minimum amount of anion or cation that causes the precipitate is known, a simple calculation will indicate the approximate concentration which will cause an incompatibility when another ionizable salt containing the same troublesome anion or cation is added to the mixture.

While studying the effects of mixtures of germicides against Gram-negative bacteria, Pittman[100] observed that certain quaternary ammonium compounds enhanced the germicidal action of Rivanol (ethoxydiamino-acridine) but not of other acridines. A corresponding enhancement of Rivanol was obtained with tyrothricin. On the other hand, anionic detergents, as well as unpurified penicillin, were found to decrease the germicidal action of Rivanol.

Tice and Pressman[101] noted that a physical incompatibility exists between negative, or anionic, gelatin (alkali treated precursor), and quaternary ammonium germicides. This was evidenced by the formation of a precipitate when the two were combined in certain ratios only. The precipitate, however, could be cleared with neutral salts. Positive or cationic gelatin (acid treated precursor), on the other hand, was found to be completely compatible with the quaternary ammonium compounds. Of particular interest was the observation that both types of gelatins were bacteriologically compatible with the cationic germicides as determined by the phenol coefficient method of evaluating bactericidal activity. The authors concluded, moreover, that there is a possibility that the gelatins augmented the germicidal efficiency of certain quaternary ammonium antiseptics.

Gershenfeld and Perlstein[102] found that by adding nonbactericidal concentrations of dioctyl-sodium-sulfosuccinate to phenol, mercuric chloride, sodium ethylmercurithiosalicylate[e] or hexylresorcinol a marked increase in the germicidal activity of the latter compounds could be obtained. However, combining dioctyl-sodium-sulfosuccinate with alkyldimethylbenzylammonium chloride resulted in a chemical incompatibility with an accompanying decrease in bactericidal efficiency of the quaternary ammonium compound.

Petroff and Schain[103] were also able to demonstrate that the addition of nongermicidal amounts of detergents such as dioctyl-sodium-sulfosuccinate or triethanolamine-tetradecyl sulfate to well-known antiseptics would materially increase the bactericidal activity of sodium *p*-toluene sulfonchloramide, chlorazodin (U.S.P. XIII), phenol, etc. The authors present an excellent theoretical consideration of the relationship of surface tension and the permeability of solutions through bacterial cell walls.

Although no mention of cationic surface-active agents (quaternaries) is made in their studies, Gershenfeld and Witlin[104] were unable to demonstrate an increase in bactericidal activity of a number of disinfectants by the addition of 39 nonbacteriostatic anionic surface tension reductants. They were also unable to show an increased rate of healing of experimental staphylococcal infections in rabbits by bactericidal solutions containing the surface tension depressants.

In studies on the germicidal action of *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride, Joslyn, Yaw, and Rawlins[85] refer to the compatibility of aqueous solutions of the compound and dilute acids and alkalies. However, they found that a precipitate would result when the quaternary ammonium germicide was combined with concentrated solutions of mineral acids. They also noted an incompatibility between the compound and soaps, as well as iodides.

Hind and Goyan[105,106] prepared a list and an analysis of buffer solutions to determine the optimal pH for ophthalmic drugs in respect to maximal stability and therapeutic efficiency. The drugs were classified into five groups and five buffer systems were recommended. As a preservative for the ophthalmic solutions against mold growth a boric acid buffer with alkyldimethylbenzylammonium chloride in a concentration of 1:50,000 was recommended. The quaternary ammonium germicide was used in solutions of Pontocaine, epinephrine, Novocaine, Benzocaine and several other local anesthetics, as well as sulfathiazole and sulfadiazine. Successful use of the preservative with the ophthalmic agents listed would indicate that the quaternary salt was compatible with most of the drugs in the concentration given.

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[e] See page 198 for trade names and distributors.

Alkyldimethylbenzylammonium chloride has also been advocated as a preservative for a drug (diethanolamine salt of 2,4-dioxo-3-iodo-6-methyltetrahydropyridine acetic acid) which is used as a contrast medium for the visualization of body cavities.[107] Tetraethylammonium chloride solutions, the new drug used in peripheral vascular disease, has been reported by Niemeyer and Green[108] to contain *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride as the sterilizing agent.

Bryson and Grace[109,110,111] inferred in several publications that alkyldimethylbenzylammonium chloride is compatible with penicillin and streptomycin. This was based on their findings of the successful use of these combinations in the aerosol therapy of respiratory diseases. The authors also presented laboratory assays indicating that a compatibility exists between the two antibiotics mentioned and the quaternary ammonium germicide. A brief account of their clinical findings will be presented in a later section of this monograph.

Fulton and his co-workers[112] determined the effects of combining a long list of germicides with dipropylene glycol with the object of preparing germicidal aerosols with maximum antibacterial activity and minimum irritating effects when the aerosols were inhaled by human subjects. Included in the list of germicides tested were several quaternary ammonium compounds on which combination with dipropylene glycol the following factors were noted: the *p*-tertiaryoctylphenoxy- and *p*-tertiaryoctylcresoxy- derivatives of ethoxyethyldimethylbenzylammonium chloride were insoluble in the organic solvent, and *N*-(higher acyl esters of colaminoformylmethyl) pyridinium chloride formed colored compounds with dipropylene glycol. While alkyldimethylbenzylammonium chloride and octadecyldimethylbenzylammonium chloride in the same study were apparently chemically and physically compatible with the latter solvent, they were reported to be very irritating in the air when in the form of an aerosol spray. The quaternary ammonium germicides which were found to be compatible with dipropylene glycol in the form of aerosols and were but mildly irritating were cetyldimethylethylammonium bromide and cetyltrimethylammonium bromide. A further consideration of these aerosols will also be made in another section of this review (page 168).

Interfering Action of Tap Waters

The Sanitary Engineering Division of the U. S. Public Health Service, Washington, D. C., prepared a statement at the request of the conference of State Sanitary Engineers meeting in Washington, D. C., May 7-8, 1947, concerning a two-year study on the properties of quaternary ammonium compounds.[113] The investigations carried out by the Public Health Service Water and Sanitation Investigations Station at Cincinnati, Ohio, involved tests on the efficiency of the quaternary ammonium germicides in regard to: (1) concentration of bactericidal agent; (2) time of contact between bactericidal agent and organism; (3) temperature of bactericidal treatment; (4) pH of the water containing the bactericidal agent; and (5) interfering substances, organic or inorganic, in the waters in which the bactericidal agents are used.

TABLE 4

Interfering Action of Tap Waters on Germicidal Properties of Quaternary Ammonium Compounds

(Report of U. S. Public Health Service, Sanitary Engineering Division, prepared for Conference of State Sanitary Engineers meeting, Washington, D. C., May 7-8, 1947.)

Compound Designated	Ppm. Required to Produce 100% Kill of *Esch. coli* in 1 Min. When Compound Is Dissolved in			Ppm. Recommended for Use by Producer
	Pure Water	Cincinnati Tap	Norwood Tap	
A	20	100	250	234
B	10	70	220	190
C*	5	500	3200	78

* Compound C differs from B in that a good detergent has been added to C. Evidently in pure water the detergent enhances the bactericidal efficiency of the active agent and in good tap waters the combination of detergent and interfering substances practically destroys all bactericidal action.

While the report indicated little concern for the first four items listed, the fifth item appeared to be a cause for some concern to the operator or user of quaternary ammonium germicides as sanitizing agents in milk pasteurizing plants and in eating and drinking establishments. Evidence was presented to show (Table 4) that 20 parts per million (ppm.) of a quaternary ammonium compound designated as "A" would produce a 100% kill of *Escherichia coli* in one minute when it was dissolved in distilled water, whereas 100 ppm. was required to give the same effect when the germicide was dissolved in Cincinnati tap water[f] and 250 ppm. in Norwood water.[g]

[f] Found to be of approximately 154 ppm. hardness as determined by soap-hardness test.

[g] Estimated as 445 ppm. hardness.

The concentration or ppm. recommended for use by the producer was 234 ppm. In the order given, these values can also be tabulated as follows: 1:50,000, 1:10,000, 1:4,000, and 1:4,270, respectively. Based upon these findings alone and on laboratory evidence in which relatively large inocula of test cultures are used, the concentration recommended by the manufacturer for use of quaternary ammonium compound "A" closely parallels the effective killing concentration obtained in the most adverse or unfavorable water (Norwood) sample that was used in the study.

Additional data reveal, however, that another quaternary ammonium compound designated as "B" gave a 100% kill in distilled water containing 10 ppm. of the germicide, 70 ppm. when in Cincinnati tap water, and 220 ppm. in Norwood water. The concentration for use recommended by the manufacturer for compound "B" was 190 ppm. It is apparent, therefore, that the margin of safety for providing 100% kill in this case is somewhat less than with compound "A." Included in this investigation were tests on a product prepared by adding a "good" detergent to quaternary "B." This material, designated as "C," gave a 100% kill in distilled water by only 5 ppm. of the compound, yet 500 ppm. were required of the same preparation to give the same effect in Cincinnati water and 3,200 or more ppm. in Norwood water. The manufacturer's recommendations for use of quaternary-detergent "C" was 78 ppm. It is of particular significance in the latter case, therefore, that the manufacturer was totally unaware of the existence of interfering substance in certain tap waters and based his formulation solely on data derived from studies conducted in distilled waters in the laboratory.

On the basis of the evidence presented in the U. S. Public Service report, the department suggested that health officers may provisionally permit the use of quaternary ammonium germicides on a limited scale and under controlled conditions where frequent bacteriological sampling could be done to determine their effectiveness. No reference was made in this or a subsequent publication[114] concerning the nature of the interfering substance or substances which are responsible for the reduction in activity of the quaternary ammonium compounds in the two waters that were tested. A reproduction of the data contained in the U. S. Public Health articles may be found in subsequent publications by McCulloch, Hauge, and Migaki.[17,115]

Ridenour and Armbruster[116,117] prepared a literary review and listed several factors which affected the properties of quaternary ammonium compounds as sanitizing agents. Their studies included the following subjects: (1) comparative efficiencies of commercial quaternaries that were on the market; (2) effect of temperature, organic matter, water mineral substances, and pH on the germicidal activity of the compounds; (3) relative resistance of various types of organisms to the bactericidal agents; and (4) chemical testing for active or "residual" quaternary ammonium germicide. In the

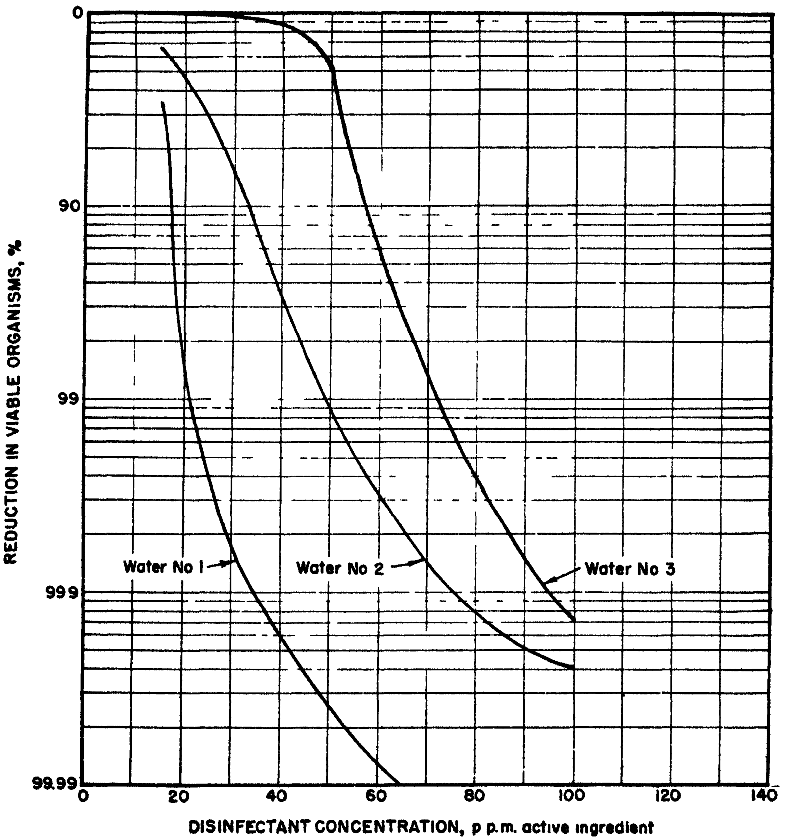

Figure 1

studies on the effects of various waters on the germicidal activity of the latter class of disinfectants a comparison was made of the activity of the compounds when tested in distilled water, a softened water from a municipal plant with a soap hardness of 80 ppm. and a moderately hard untreated water with a soap hardness of 275 ppm. Using *E. typhosa* as the test organism, Ridenour and Armbruster were able to demonstrate that in order to obtain a 99.9%

kill the required concentrations of the same compound, tested under the same conditions, were 34, 75, and 95 ppm. respectively of the three different waters. A reproduction of these data is presented in Figure 1.

Ridenour and Armbruster extended the studies originally reported by the U. S. Public Health Service on the interfering action of certain tap water on quaternary ammonium compounds by investigating the factor or factors that are responsible for the differences in results obtained in various waters. The factors studied included pH, organic matter, and mineral salts. Their data, as well as the reported finding of previous investigators, ruled out pH as the contributing agent responsible for the adverse action on quaternary ammonium activity. They were able to demonstrate, moreover, that the organic matter content of their test waters was negligible and

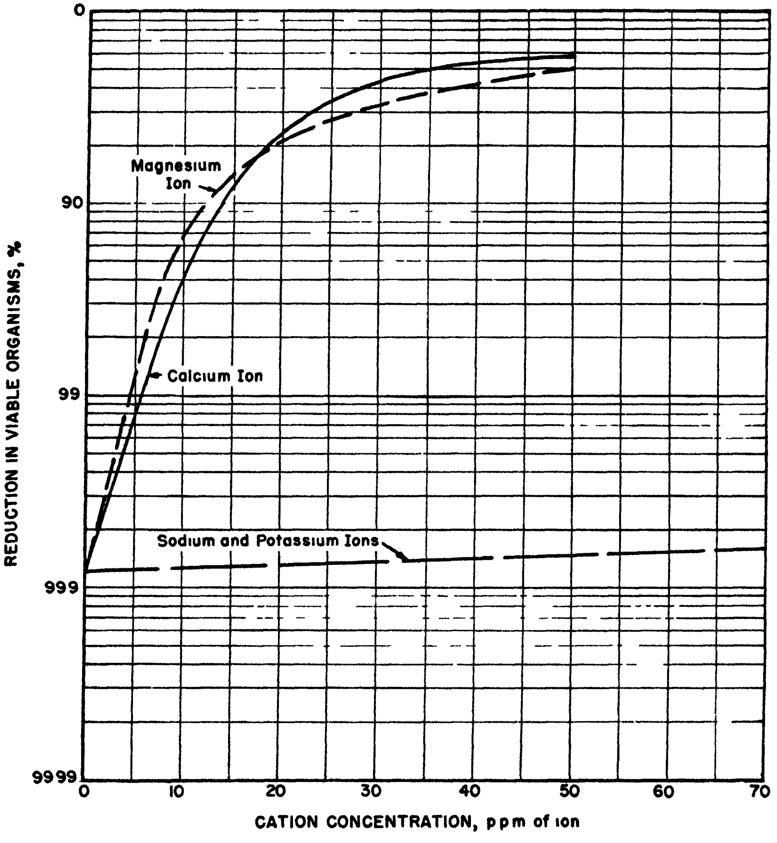

Figure 2

also could be eliminated as the cause of the interfering action on these compounds.

Their attention was directed, therefore, to one or more of the common mineral water ions that are normally present in tap waters. This study was carried out by adding separately to the quaternary ammonium solutions different common water ions such as the cations, magnesium, calcium, sodium, and potassium. The anions tested were sulfates, nitrates, chlorides, normal phosphates, and carbonates. The water used in the study was free of organic matter and the pH was approximately neutral with the exception of the tests in which phosphates and carbonates were used. A reproduction of their results on these studies is presented in Figures 2 and 3.

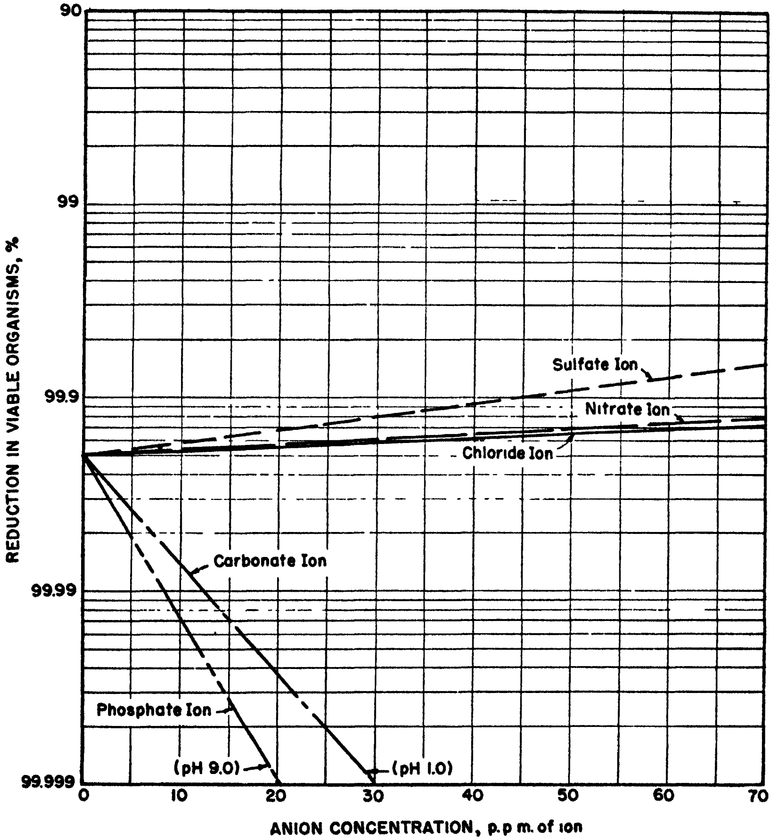

Figure 3

Examination of their data will reveal that the cations, calcium, and magnesium in the test solutions will materially reduce the germicidal activity of a quaternary ammonium germicide against *E. typhosa.* A decrease in bacterial count from the original 99.9% to 50% occurred with an increase of calcium or magnesium cations from 0 to 40 ppm. Sodium and potassium cations, as well as the several anions tested, failed to have any adverse effect on the germicidal activity of the quaternary ammonium compound.

The report of the adverse effects of certain tap waters upon quaternary ammonium activity did not originate with the U. S. Public Health Service or Ridenour and Armbruster, for a review by Hanne[118] in 1936 on the subject of testing disinfectants makes reference to the actions of a quaternary ammonium germicide against *E. coli* when tested in distilled water as compared to tests in tap water. Reference was made to the effect that while a 1:10,000 of alkyldimethylbenzylammonium chloride would destroy the Gram-negative organism in 8 minutes in distilled water, a time period of 16 minutes was required to produce the same results when tap water was used as the diluent. A concentration of 1:5,000 of the quaternary ammonium germicide in tap water was necessary to destroy *E. coli* in 8 minutes.

TABLE 5

INTERFERING ACTION OF TAP WATER* ON GERMICIDAL PROPERTIES OF QUATERNARY AMMONIUM COMPOUNDS AGAINST *E. Typhosa*—"HOPKINS"

COMPOUND	DILUTION	TRANSFER MINUTES			PHENOL COEFFICIENT	
		5	10	15		
Alkyldimethyl-benzylammonium chloride	1: 5,000	+	−	−	$\frac{5,000}{90}$	55 5
	1:10,000	+	+	+		
	1:15,000	+	+	+		
	1:20,000	+	+	+		
	1:25,000	+	+	+		
N-(acyl-colamino-formyl-methyl) pyridinium chloride	1: 1,000	−	−	−	$\frac{2,000}{90}$	22 2
	1: 3,000	+	+	−		
	1: 6,000	+	+	+		
	1: 9,000	+	+	+		
	1:12,000	+	+	+		
Phenol standard	1: 80	−	−	−	Divisor 90	
	1: 90	+	−	−		
	1:100	+	+	−		

*Tap water of approximately 350 ppm. soap-hardness.

Studies carried out by the writer confirm the reported findings on the adverse effects of certain tap waters upon the germicidal activity of several commercial quaternary ammonium compounds against Gram-negative bacteria (*E. coli, E. typhosa*). The results of phenol coefficient tests on two quaternary ammonium compounds dissolved in a soap-hardness water of 350 ppm. against *E. typhosa* are presented in Table 5. Although not indicated in the table, phenol coefficient values of approximately 250 to 275 are obtained on these quaternary ammonium germicides when the latter are tested in distilled water at 20°C.

TABLE 6

Action of Tap Water* on Germicidal Properties of Quaternary Ammonium Compounds Against *S. Aureus*—209

Compound	Dilution	Transfer Minutes			Phenol Coefficient	
		5	10	15		
Alkyldimethyl-benzylammonium chloride	1:15,000	−	−	−	$\frac{18,000}{60}$	300
	1:18,000	+	−	−		
	1:21,000	+	+	+		
	1:24,000	+	+	+		
	1:27,000	+	+	+		
N-(acylcolamino-formylmethyl) pyridinium chloride	1: 1,000	−	−	−	$\frac{2,000}{60}$	33.3
	1: 2,000	+	−	−		
	1: 3,000	+	+	−		
	1: 4,000	+	+	−		
	1: 5,000	+	+	−		
p-tertiaryoctyl-phenoxyethoxy-ethyldimethyl-benzylammonium chloride	1:15,000	+	−	−	$\frac{21,000}{60}$	350
	1:18,000	+	−	−		
	1:21,000	+	−	−		
	1:24,000	+	+	−		
	1:27,000	+	+	+		
9-octadecenyl-dimethylethyl-ammonium bromide	1:15,000	−	−	−	$\frac{18,000}{60}$	300
	1:18,000	+	−	−		
	1:21,000	+	+	−		
	1:24,000	+	+	−		
	1:27,000	+	+	−		
Cetylpyridinium chloride	1:15,000	−	−	−	$\frac{21,000}{60}$	350
	1:18,000	−	−	−		
	1:21,000	+	−	−		
	1:24,000	+	+	+		
	1:27,000	+	+	+		
Phenol standard	1:60	+	−	−	Divisor	60
	1:70	+	+	−		
	1:80	+	+	+		

*Tap water of approximately 350 ppm. soap-hardness. All dilutions of germicides prepared in tap water.

Since several of the quaternary ammonium compounds are available on the market as medicinal or pharmaceutical germicides, it appeared desirable to determine whether or not the "hardness" of waters would also influence the activity of the compounds against Gram-positive bacteria (*Streptococcus pyogenes*, *S. aureus*, *Diplococcus pneumoniae*, etc.) which are commonly encountered in surgical or skin infections. For this purpose a standard strain (209) of

S. aureus was chosen as the test organism for a phenol coefficient evaluation of several quaternary ammonium disinfectants in the presence of tap water of 350 ppm. soap-hardness. The results of this study are presented in Table 6.

Examination of the data will reveal the unexpected or unusual results that were obtained with all but one of the series of quaternary ammonium compounds tested. In general, unlike the results obtained when Gram-negative organisms, *E. coli* or *E. typhosa,* were used in the presence of quaternary and "hard" waters the germicidal activity was not impaired in the same water when the Gram-positive culture of *S. aureus* was used. Subsequent studies, in which synthetically prepared hard water containing calcium and magnesium cations was used as the diluent for the germicides, confirmed the latter observations on the inability of the ions to interfere with

TABLE 7

EFFECTS OF VARYING CONCENTRATIONS OF HARD WATERS (ESTIMATED AS $CaCO_3$ SOAP-HARDNESS) UPON THE GERMICIDAL ACTIONS OF QUATERNARY AMMONIUM COMPOUNDS* AGAINST *E. Typhosa*—"HOPKINS"

HARDNESS PPM.	DILUTION	TRANSFER MINUTES			PHENOL COEFFICIENT	
		5	10	15		
50 Ppm.	1: 5,000	–	–	–	$\frac{15{,}000}{85}$	176
	1:10,000	–	–	–		
	1:15,000	+	–	–		
	1:20,000	+	+	–		
	1:25,000	+	+	–		
100 Ppm.	1: 5,000	–	–	–	$\frac{12{,}000}{85}$	141
	1:10,000	–	–	–		
	1:15,000	+	+	–		
	1:20,000	+	+	+		
	1:25,000	+	+	+		
200 Ppm.	1: 5,000	–	–	–	$\frac{10{,}000}{85}$	117
	1:10,000	+	–	–		
	1:15,000	+	+	+		
	1:20,000	+	+	+		
	1:25,000	+	+	+		
250 Ppm.	1: 5,000	–	–	–	$\frac{7{,}000}{85}$	82
	1:10,000	+	+	–		
	1:15,000	+	+	+		
	1:20,000	+	+	+		
	1:25,000	+	+	+		
Phenol standard	1: 80	–	–	–	Divisor	85
	1: 90	+	+	+		
	1:100	+	+	+		

*Alkyldimethylbenzylammonium chloride used as test compound with hard water as a diluent throughout.

germicidal action of the quaternary salts upon *S. aureus*. The single exception in the series of quaternary ammonium germicides tested is the *N*-(acylcolaminoformylmethyl) pyridinium chloride salt. The activity of this derivative was reduced by hard water against *S. aureus* approximately to the extent as when *E. typhosa* was used as the test organism.

A study was made to determine the relationship of the degree of hardness of water and the interfering action on the germicidal activity of a quaternary ammonium compound against *E. typhosa*. Alkyldimethylbenzylammonium chloride was selected as the test compound in water hardness solutions of 50, 100, 200, and 250 ppm. The results of this study are given in Table 7. It is evident from the data presented in the table that there is a direct correlation between the degree of water hardness ($CaCl_2$) and the germicidal activity of the quaternary ammonium compound against a Gram-negative organism.

Since various salts are known to enhance the germicidal action of quaternary ammonium compounds, based upon the influence of pH, it appeared desirable to determine the effects of the addition of several of these compounds to solutions of the germicides prepared in hard water. Several of the agents tested were included in the series as possible "water-softening" agents. Tables 8 and 9 present a tabulation of the degree of turbidity, pH, determination of soap-hardness, and phenol coefficient values on the several solutions using alkyldimethylbenzylammonium chloride against *E. typhosa* at 20°C.

Examination of the data reveals that the addition of trisodium phosphate in a concentration as low as 0.01% to the quaternary ammonium compound in hard water will materially enhance the germicidal activity of the compound against *E. typhosa*. An optimal concentration of 0.05% of the alkaline salt appears to bring the bactericidal activity of the quaternary ammonium compound in the range of potency expected when the latter is tested in distilled water. These results suggest the possible use of alkaline detergents or sequestering agents in combination with quaternary ammonium sanitizing agents in certain areas where the waters have an adverse effect on the activity of the latter compounds. A consideration of the use of "quaternary-sanitizers" or "quaternary-detergent" formulations, which have certain advantages over the use of quaternary ammonium compounds alone, will be considered in subsequent sections of this review (pages 171, 177).

In comparing the "speed" of germicidal activity of alkyldimethyl-

TABLE 8

ENHANCEMENT OF GERMICIDAL ACTIVITY OF QUATERNARY AMMONIUM COMPOUNDS* IN THE PRESENCE OF HARD WATER** AGAINST GRAM-NEGATIVE BACTERIA

COMPOUND(1%)	TURBIDITY	pH	SOAP HARDNESS (Ppm.)	QUATERNARY DILUTION	TRANSFER MINUTES			PHENOL COEFFICIENT
					5	10	15	
Sodium hexametaphosphate	2+***	9 25	143	1: 5,000	+	–	–	55
				1:10,000	+	+	+	
				1:15,000	+	+	+	
Trisodium phosphate	1+	9 50	116	1: 5,000	–	–	–	166
				1:10,000	–	–	–	
				1:15,000	+	–	–	
Sodium borate	0	8 95	163	1: 5,000	–	–	–	83
				1:10,000	+	+	–	
				1:15,000	+	+	–	
Sodium tetraphosphate	0	8 55	51	1: 5,000	–	–	–	140
				1:10,000	–	–	–	
				1:15,000	+	+	–	
Sodium nitrite	0	8 40	139	1: 5,000	–	–	–	111
				1:10,000	+	–	–	
				1:15,000	+	+	–	
Phenol standard (*E. typhosa*—"Hopkins")	......	....	.	1: 80	–	–	–	Divisor 90
				1: 90	+	–	–	
				1:100	+	+	–	

*** Alkyldimethylbenzylammonium chloride.**

**** Estimated as 368 ppm. soap-hardness.**

***** 2+, 1+ to 0 indicates marked to slight and no turbidity.**

TABLE 9

EFFECTS OF LOW CONCENTRATIONS OF TRISODIUM PHOSPHATE AND SODIUM TETRAPHOSPHATE ON THE INTERFERING ACTION OF HARD WATER* UPON THE GERMICIDAL ACTIVITY OF A QUATERNARY AMMONIUM COMPOUND**

DETERGENT CONCENTRATION	QUATERNARY DILUTION	TRANSFER MINUTES			DETERGENT CONCENTRATION	QUATERNARY DILUTION	TRANSFER MINUTES		
		5	10	15			5	10	15
Trisodium phosphate 0.01%	1: 5,000	–	–	–	Sodium tetraphosphate 0.01%	1: 5,000	+	–	–
	1:10,000	+	–	–		1:10,000	+	+	+
	1:15,000	+	+	+		1:15,000	+	+	+
	1:20,000	+	+	+		1:20,000	+	+	+
	1:25,000	+	+	+		1:25,000	+	+	+
Trisodium phosphate 0.025%	1: 5,000	–	–	–	Sodium tetraphosphate 0.025%	1: 5,000	+	–	–
	1:10,000	–	–	–		1:10,000	+	+	+
	1:15,000	+	–	–		1:15,000	+	+	+
	1:20,000	+	+	–		1:20,000	+	+	+
	1:25,000	+	+	+		1:25,000	+	+	+
Trisodium phosphate 0.05%	1: 5,000	–	–	–	Sodium tetraphosphate 0.05%	1: 5,000	–	–	–
	1:10,000	–	–	–		1:10,000	+	+	+
	1:15,000	–	–	–		1:15,000	+	+	+
	1:20,000	–	–	–		1:20,000	+	+	+
	1:25,000	+	+	+		1:25,000	+	+	+
Phenol standard (*E. typhosa*—"Hopkins")	1: 80	–	–	–					
	1: 90	+	–	–					
	1:100	+	+	–					

* Estimated 350 ppm. soap-hardness.

** Alkyldimethylbenzylammonium chloride.

benzylammonium chloride upon *E. coli* in the presence of various waters, Johns[119,120] also considered that the hardness of certain waters is the factor which is responsible for the reduction in activity of the quaternary ammonium compound. Examples given indicate that while 50 ppm. of the germicide will destroy the test organism in distilled water, a concentration of 100 ppm. of the same compound is required to produce the same effect in a water of 60 ppm. hardness. In a well water of 162 ppm. hardness the concentration of the germicide had to be increased to 200 ppm. to give a comparable degree of destruction of the Gram-negative organism. In the same study, but using the Gram-positive organism *S. aureus,* the germicidal activity of the quaternary ammonium compound was not reduced by the various hard waters from that noted when distilled water was used as the diluent. These findings are in accord with the data obtained by the writer.

A brief note by Barber and Harris[121] also indicates that hard water greatly reduces the germicidal effectiveness of quaternary ammonium compounds.

In contrast to the belief that the calcium and magnesium ions in hard waters are solely responsible for the interfering action on quaternary ammonium germicides, Mueller and Seeley[122] have recently reported that waters with appreciably high iron content would reduce the activity of the compounds against certain bacteria. The inference was, furthermore, given that waters of relatively high degrees of hardness were found to have little if any adverse effects on the activity of some quaternary ammonium compounds used in their investigations. A published account of their findings will be a subject of extreme interest to other investigators on this class of germicidal agents.

CHEMICAL ASSAYS

Hartley and Runnicles,[123] in collaboration with N. K. Adam, developed a colorimetric method for determining quaternary ammonium cations. The test is based on the fact that the color of sulfonphthalein indicators is displaced to the alkaline side by paraffin-chain cations (quaternaries) and the change can be measured qualitatively. A concentration of N/1000 cetylpyridinium ion will change the normal alkaline purple of bromophenol blue to a pure blue. The color change is used as the end-point for the direct titration of a long-chain alkyl anion against a long-chain alkyl cation resulting in the formation of an insoluble salt. When the long-chain cation is removed the alkaline purple color is restored. By this procedure N/1000 cetylpyridinium or trimethylammonium ions can be titrated against N/1000 cetyl sulfate, or cetane sulfonate, to within 1%. Less accuracy may be expected when testing concentrations of quaternaries lower than N/1000.

In conducting the test, 2 ml. samples of quaternary ammonium solution are placed in a 15 ml. test tube. One-tenth milliliter of M/5000 bromophenol blue, made slightly alkaline with ammonium hydroxide, is added to the tube and the standardized anionic solution run in from a microburet. The titration tube is placed in a comparator between tubes containing mixtures of standards 3% on either side of the end-point. The solution is then titrated to a color mid-way between the purple and blue colors of the standards.

A colorimetric assay method for the estimation of quaternary ammonium compounds in solution was also described by Auerbach.[124] The method consists of forming the quaternary ammonium-dye salt by the addition of bromothymol blue or bromophenol blue in carbonate solution. The color is extracted with ethylene dichloride and the intensity of the extract is measured in a photoelectric colorimeter. The concentration of quaternary ammonium salt is readily calculated by means of a factor previously derived from a standard solution of the ammonium salt. Since some workers found it difficult to clarify or dry the ethylene dichloride dye solution without affecting its color intensity, slight modifications in the test procedure were made by Auerbach.[125] These consisted of: (1) using benzene as the solvent in place of ethylene dichloride; (2) reducing the amount of sample from 200 μg. to 50 μg.; and (3) using a light filter transmitting 540 mμ instead of one transmitting at approximately 600 mμ. Details of the test procedure as it is used at present are as follows:

Fifty milliliters of water containing 0.050 to 0.075 mg. of quaternary salt is added to a 125 ml. separatory funnel. Five milliliters of 10% sodium carbonate, 1 ml. of aqueous 0.04% bromophenol blue and exactly 10 ml. of benzene are added to the separatory funnel in the order given. (The indicator solution should be prepared on the day it is to be used by dissolving 40 mg. of bromophenol blue in 100 ml. of water containing 1 ml. of N/10 sodium hydroxide). The contents of the funnel are shaken gently for 2 to 3 minutes. The layers are allowed to separate roughly and the contents of the funnel swirled. After standing for several minutes, or until well separated, the aqueous layer is removed from the funnel and a portion of this solution used to rinse a 15-ml. centrifuge tube. The remainder of the aqueous phase is discarded. The colored benzene layer is then run into the tube. The tube is stoppered with a clean diaphragm stopper and centrifugated for a few minutes to clarify. The clear solution is then placed in a photoelectric colorimeter tube and read through a glass filter transmitting light at 600 mμ. The limit of error of this test procedure is about ±2%.

Wilson[126] and Harris[127] have made use of the Auerbach procedure for the determination of quaternary ammonium germicides in various classes of food products including fruit juices, bottled sodas, mayonnaise, salad dressing, milk, beer, sirup, dog biscuits, pickles, and relish.

Colichman[128] described a modification of Auerbach's method in which the color intensity is measured directly without extracting the colored complex with an organic solvent. A photoelectric colorimeter is used with a proper filter to record the density of the color. The method is applicable for routine commercial analyses of concentrations of quaternary salts in solutions of 20 ppm. or higher.

Using the principles of the Auerbach method for the colorimetric determination of quaternary ammonium solutions, Brooks and Hucker[129] describe a test for the germicides which consists of titrating with a 0.04% solution of bromophenol blue buffered to pH 4.6 in the presence of ethylene dichloride. DuBois and Dibblee,[47,48] however, found that the latter test was unsuitable for the determination of quaternary ammonium compounds in milk. Preference was given to the use of the Hartley and Runnicles method for estimating the concentration of the germicides in studies on the bacterial counts of milk samples. They proposed, moreover, a rapid qualitative test for the detection of quaternary ammonium compounds in solutions. This test is a modification of the Hartley-Runnicles procedure and consists of adding 0.7 ml. of the indicator solutions to 2 ml. of the test solution. The indicator consists of two parts of 0.01% bromophenol blue made slightly alkaline with ammonia and five parts of 1:2500 Duponol PC. A pure blue solution results when the quaternary ammonium concentration is approximately 1:7000, otherwise it is of purple color. Higher or lower concentrations of the germicides can be determined by increasing or decreasing the amount of Duponol in the test.

A rapid method for analyzing surface-active agents has been described by Epton[130] which makes use of the principle that while the chloride of the basic dyestuff, methylene blue, is insoluble in chloroform, the alkyl sulfate and related salts are freely soluble in the solvent. These may, therefore, be extracted quantitatively from aqueous solutions by chloroform provided that the chain length of the organic portion of the molecule is sufficiently large. However, the extraction is inhibited if the aqueous phase contains a cationic surface-active compound more than equivalent to the anionic material present in the solution. The explanation given for this reaction is that the colorless stoichiometric compound formed by combining the cationic-quaternary ammonium salt and the anionic compound is more soluble in chloroform and is extracted in preference to the methylene blue salt. The blue color will, therefore, remain in the upper aqueous layer. Details of the application of this reaction for determining concentrations of quaternary ammonium compounds are presented in the report by Epton.[130]

Simple, although not extremely accurate, procedures for determining quaternary ammonium concentrations are the test-paper methods described by Stone,[131] Marshall, and Portley.[132] By immersing a strip of dye-impregnated paper in an aqueous solution of the germicides, a change of color occurs which is compared with a color chart indicating the approximate amount of quaternary ammonium salt in the test. With little practice, the authors state that concentrations of 0, 100, 200, and 300 ppm. of germicide can be easily and quickly determined. With the Stone-Marshall papers the color ranges given vary from no change to lavender, gray, and green. A definite green tint indicates approximately 200 ppm. of quaternary in the solution which is the acceptable concentration for most sanitizing purposes. The Portley papers, on the other hand, vary from light yellow to orange and red. The latter color indicates an effective concentration of quaternary ammonium in solution.

Flotow[133] designed a test procedure that involved the precipitation of the quaternary ammonium salt with an excess of potassium dichromate and the free dichromate was then determined iodometrically. As an alternative he proposed a method in which a colorimetric estimation was made of a solvent extractable permanganate formed between the latter and the high molecular cations.

Bartlett[134] more recently described a test method for the estimation of quaternary ammonium germicides in solution involving an entirely different reaction than that carried out in the colorimetric tests. This method may be outlined briefly as follows:

Approximately 1 gm. of potassium dichromate is added to 5 ml. of the quaternary ammonium solution (85°F. ±10°) in a calibrated test tube. The precipitate that is formed is mixed by gently inverting the tube 10 times. To the tube is then added 0.5 ml. of a 0.75%

solution of Triton X-100[h] and the contents of the tube mixed by inverting four times and the addition of Triton continued drop by drop. After each addition the tube is inverted and the process continued until the solution is clear or black printed numbers 1- to 2-mm. high can be read through the solution. The dilution of quaternary ammonium germicide in the solution is then calculated on the basis of the molecular weight of the compound and the number of drops of Triton required to clear the solution. This test is claimed to be accurate to within 20 ppm. of the germicides.

A volumetric precipitation method for the determination of quaternary ammonium compounds has been developed by Auerbach.[135] A volume of solution representing approximately 0.5 gram of the quaternary is diluted in distilled water to 100 ml. Exactly 10 ml. of the solution is transferred to a 100-ml. flask to which is added 5 ml. of buffer solution (260 gram sodium acetate and 280 ml. 30% acetic acid to make 1000 ml.) and 50 ml. of 0.020 N/1 potassium ferricyanide. The solution is then diluted to 100 ml. and allowed to stand for one hour. The mixture is filtered through paper and the first 20 ml. discarded. To the next 50 ml. is added 5 ml. of 10% potassium iodide solution and 5 ml. of dilute hydrochloric acid. Ten milliliters of 10% zinc sulfate solution is then added and titrated with N/100 sodium thiosulfate, using starch as the indicator. The weight of the quaternary ammonium salt is calculated by the formula (50 ml. N/100 $Na_2S_2O_3)_x$, a factor representing the molecular weight of compound tested. The amount of germicide found will be not less than 97% nor more than 103% of the original as calculated to the anhydrous substance.

A review of other methods that can be used for the chemical estimation of quaternary ammonium germicides may be found in publications by DuBois.[136,137,138] In one of these reviews DuBois[137] described the details of a precipitation method which consists of the addition of iodine-potassium iodide solution to the quaternary ammonium solution. In the presence of starch the color change is reported to be from yellow through various shades of green and sharply to blue. This change in color occurs gradually and varies with each quaternary ammonium compound tested.

Hager, Young, Flanagan, and Walter[139] determined quaternary ammonium concentrations by reactions which converted the latter to triiodides. Their qualitative procedure involves the addition of five drops of 5% sodium hypochlorite solution and 0.5 ml. of glacial acetic acid to 25 ml. of the test solution. A piece of starch iodide test paper is then added to the solution and the color of the paper noted. If the concentration of the quaternary ammonium solution is approximately 0.1% the paper will appear blue. Concentrations in the range of 0.5% will result in a bright yellow paper. In between the concentrations given, the authors describe the color variations to be from blues to greens through browns to yellow. In addition to the starch iodide method just outlined, the same investigators described a turbidimetric procedure for quaternary ammonium concentration in which potassium iodide is added to an acidified solution of the germicide. The resulting precipitate will vary in color from yellow to orange and reddish-brown.

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[h] Alkylated aryl poly-ether alcohol.

Harper, Elliker and Moseley[140] presented a test method for testing quaternary ammonium concentrations in which "eosin yellowish" (tetrabromofluorescein) is allowed to react with the germicide to form a bright red precipitate. The anionic surface-active agents will disrupt the chemical combination by replacing the eosin salt formed and thereby result in a loss of color. The method can be incorporated in a paper test formulation. The authors claim that their test procedure will account for a quaternary ammonium compound even though the germicidal activity is partly or completely inhibited by organic matter such as 10% milk.

Mention may also be made of a method described by DuBois[141] as the argentimetric procedure using eosin or dichlorofluorescein for the determination of quaternary ammonium concentrations. While the determination of halides by titration with silver nitrate has been widely used in chemistry using potassium chromate as the indicator in the presence of the high molecular quaternary ammonium salts, there is a tendency for these precipitates to obscure the end-point. For this reason preference was given by DuBois to the use of eosin and dichlorofluorescein as indicators that give easily detectable end-points.

While most of the chemical methods for determination of quaternary ammonium concentrations give reliable and accurate results under carefully controlled titration procedures, there still is needed a simple test that is rapid and requires no special apparatus. Furthermore, of particular importance to public health officers is a test that not only includes the latter requirements but also provides a reasonably accurate estimation of the bactericidal active concentration of a quaternary ammonium germicide in the presence of inorganic and, in particular, organic matter.

Gain and Lawrence[142] recently described a rapid test method for estimating "Use Dilution" concentrations of quaternary ammonium germicides. The procedure involves the use of a single tube to which is added 1 ml. of the unknown solution and one drop of undiluted, normal horse serum. The contents of the tube are shaken and the presence or absence of turbidity noted within 30 seconds. Complete absence of turbidity at this time indicates a concentration of less than 166 ppm. (1:6000) active quaternary ammonium germicide, which is the usual minimal limit recommended for an effective sanitizing concentration of most quaternary ammonium germicides. As an example of the results that may be expected in adding one drop of serum to 1-ml. quantities of various concentrations of a quaternary salt, Gain and Lawrence presented the following scheme for recording their test.

1,000 ppm. (1:1000)	++++	Marked turbidity
500 ppm. (1:2000)	++++	Marked turbidity
250 ppm. (1:4000)	++	Moderate turbidity
166 ppm. (1:6000)	+	Trace of turbidity
125 ppm. (1:8000)	——	No turbidity

Evidence was also presented in the study to show that, whereas a chemical test would give the theoretical amount of quaternary present in a solution containing protein substances, the turbidimetric procedure, using horse serum, indicated that the quaternary ammonium compounds were absorbed by the protein subtances and probably inactivated accordingly. The possible use of serum in a test for quaternary ammonium concentrations was prompted by evidence in the literature which indicated that "reversal" of quaternary ammonium action on bacteria does not occur when the germicide is used in concentrations approaching 1:6000 or more. In line with this theory, it was postulated that the concentrations given would definitely disorganize, coagulate, or precipitate the proteins in horse serum, similar to the action of quaternary ammonium germicides on other protein substrates, including bacterial cells. Table 10 presents the data obtained in a series of tests using the serum precipitation procedure.

Flanagan, Drennen, and Goetchius[143] described a precipitation test analogous to the serum test of Gain and Lawrence in which a nondetergent agent, sodium salt of a condensed aryl sulfonic acid, was used instead of a protein reagent to precipitate the quaternary ammonium germicide from solutions. The Flanagan test method was designed to give a flocculent precipitate when appropriate amounts of the detergent are added to 1:4000, or "use dilution" concentrations of the quaternary ammonium germicide under examination. As in the Gain—Lawrence procedure the Flanagan test is unique

TABLE 10

COMPARISON OF HARTLEY-RUNNICLES (DUBOIS) COLORIMETRIC AND HORSE SERUM TURBIDIMETRIC METHODS FOR DETERMINATION OF QUATERNARY AMMONIUM COMPOUNDS

(Gain and Lawrence, *Science*, **106**: 525, 1948)

QUATERNARY AMMONIUM (1:1,000 + inactivating agents)	COLORIMETRIC (ml. Duponol "C")	TURBIDIMETRIC (1 drop serum)
Soap		
1:1,000 (unfiltered)	1.5	....
1:1,000 (filtered)	0.3	0
1:100 (unfiltered)	No reaction*	..†
1:100 (filtered)	No reaction	0
Santomerse‡		
1:1,000 (unfiltered)	1.0	....
1:1,000 (filtered)	0.3	0
1:100 (clear)	No reaction	0
Duponol "C"‡		
1:1,000 (unfiltered)	Off color§	....
1:1,000 (filtered)	0.3	0‖
1:100 (clear)	No reaction	0
Egg albumen		
1:1,000 (clear)	2.4	+++¶
1:100 (unfiltered)	Off color	....
1:100 (filtered)	2.0	++
Evaporated milk		
1:100 (unfiltered)	2.3	....
1:100 (filtered)	1.6	++
1:10 (unfiltered)	1.8	....
1:10 (filtered)	0.6	Trace
Orange concentrate—undiluted		
(unfiltered)	Off color	....
(filtered)	Off color	Trace
Ice cream		
1:10 (unfiltered)	1.5	....
1:10 (filtered)	0.7	Trace
Horse serum		
1:10 (unfiltered)	2.5	....
1:10 (filtered)	1.5	Trace
1:5 (unfiltered)	Off color	....
1:5 (filtered)	3.2	0
1:2 (unfiltered)	Off color	....
1:2 (filtered)	Off color	0
	Control	
Quaternary		
1:1,000	4.2	++++
1:2,000	2.3	++++
1:2,000 (filtered)	2.0	++++
1:4,000	1.2	++
1:6,000	0.7	0

*** No reaction—4.2 ml. or more of Duponol reagent results in no change in color.**

† ....—solution turbid; test not possible.

‡ See page 198 for chemical names and distributors.

§ Off color—dye used in test reacts with color in agent.

‖ 0—no quaternary demonstrable.

¶ ++ to ++++—moderate to marked turbidity.

in that it is a rapid one-tube, one-reagent test. The serum test, however, has the advantage in that it can be applied without modification in the testing of the majority of the commercially available quaternary ammonium germicides. The detergent-precipitation test must bear some modification in its application to the four general classes of quaternary ammonium compounds.

5. Physical Properties

SURFACE TENSION

Hornung,[144] and later Taub and his co-workers,[56,57,58] first intimated that the quaternary ammonium type of compounds would lower the surface tension of solutions. In their studies mention was made of the fact that use of certain derivatives would produce "foaming" and their use was therefore advocated for the disinfection and cleaning of inanimate objects.

Adam and Shute[76] studied the anomalies that are encountered in the surface tensions of dilute solutions of quaternary ammonium compounds. They noted that, in solutions so dilute that ionic micelles are not present in appreciable amounts, the final tension is not reached for at least a week. If the solutions are strong enough to contain micelles the final tension is reached much more quickly. By adding an inorganic salt to the dilute solutions, the fall in tension may be reached at once. In studies on cetylpyridinium bromide a sharp distinction was noted between solutions over 0.04% and those below this concentration. The stronger solutions all had a tension of approximately 37 dynes per cm. which remains fairly constant. The more dilute solutions, on the other hand, will at first have a high surface tension which will subsequently fall to almost the same value as the stronger solutions. Dilutions of 0.01% or less require at least a week to attain their final tension.

Adam and Shute observed another phenomenon in studies with cetyltrimethylammonium bromide. Solutions less than 0.045% attained a final surface tension of about 25 dynes per cm., whereas stronger solutions never went lower than 30 to 31 dynes per cm. They were unable to offer an explanation for this difference. The chain length of the alkyl group was also found to influence surface tension. A concentration of N/100 of the dodecyl (C_{12}) compound is required to produce a final surface tension at once, as compared to the same effect produced by N/1000 of the cetyl (C_{16}) derivative. The observations of Adam and Shute may explain, in part, the anomalies or wild "pluses" that are frequently encountered in testing the quaternary ammonium germicides in the phenol coefficient tests. It may be in order, therefore, to add an inorganic salt to dilute solutions of the compounds to accelerate the fall in surface tension to its permanent level before testing them *in vitro.*

From the results obtained in studies on surface-active depressants, Gershenfeld, Milanick and Perlstein[145,146] concluded that the reduced surface tension does not explain the bactericidal action of the compounds. More important are the factors of the nature of the chemicals involved; the hydrogen ion concentration; and the type of organism (Gram-positive and Gram-negative) against which they are acting.

In comparing the antibacterial activities of several quaternary ammonium sulfonamides Lawrence and Goetchius[52] have also found that the low surface tension of solutions of these compounds is not the only factor responsible for their high germicidal activity. The degree of germicidal action is in part dependent upon the structural or chemical configuration of the compound involved.

Hoogerheide[147] measured the surface tension of varying concentrations of cetyltrimethylammonium bromide and found only a slight variation between 0.1% and 2.0% of the solutions (41.5 and 38.9 dynes per cm.). He considered, however, that the lowering of surface tension is a valuable property inherent in antiseptic solutions due to their ability to penetrate minute crevices and thereby effect intimate contact between the germicide and the microorganisms.

Albert,[148] on the other hand, advanced the theory that surface-activity is a property that promotes germicidal activity in disinfectants and is lacking in most bacteriostatic agents. Using alkyldimethylbenzylammonium chloride as an example, he assumed that the compound congregates at a boundary due to its peculiar two-endedness of the molecule. This bears a powerful "polar" or "head" group (NH_2) at one end of the long axis while the rest of the molecule constitutes the "nonpolar" or "tail" group, which is simply made up of carbon and hydrogens or halogens. The longer and heavier it is, the greater the surface-activity. Reference is made in the article to Berczellers'[149] observations that the phenols showing the greatest antibacterial effects were correspondingly the most surface-active. Further support to Albert's hypothesis is given by the classification of the phenols by Suter.[150] Examination of his data reveals that the bactericidal properties of phenols increase in proportion as the molecules assume a definite "head" and "tail" arrangement. With the addition of an alkyl group the activity of a phenol increases until the length of the chain approaches five carbon atoms, beyond which there is a drop in activity.

Miller and his co-workers,[151] and later Rahn,[91] postulated that certain quaternary ammonium germicides deposit an invisible film on the hands or on the outer layer of clumps of bacteria. This film tends to retain living bacteria underneath it and may retard the diffusion of dissolved molecules to the center of clumps of organisms. The inner surface of the film has a low bactericidal power due to orientation of the molecule to the organophilic surface, whereas the outer or germicidal NOH group is directed toward the outside.

While studying the effects of primary, secondary, and tertiary alcohols for their germicidal power against bacteria, Cowles[152] noted a correlation in the length of the carbon chains and surface-activity. He found that an increase in the carbon chain was accompanied by an increase in surface tension. The close relationship between the germicidal action and surface-activity implied a biological relationship between these properties. This suggested, furthermore, that organic germicides may primarily depend upon adsorption and secondarily upon toxicity to bacterial protoplasm.

In studies on the bacteriostatic actions of various wetting agents upon the growth of the tubercle bacillus, Freedlander[153] concluded that the superiority of alkyldimethylbenzylammonium chloride over anionic and nonionic wetting agents was not due entirely to its ability to reduce surface tension. He noted that the surface tensions of the three types of wetting agents were approximately the same in all the compounds tested, and concluded, therefore, that the superior action of the cationic compound (alkyldimethylbenzylammonium chloride) was probably inherent in its molecular structure.

Taft[154] studied the effects of alkyldimethylbenzylammonium chloride on members of different phyla of invertebrates. Differences in the reaction of the organisms to the germicide were not attributed to phylogenetic differences but rather to the type of protective covering of the animal. The action of the germicide was believed to be the result of its ability to lower the surface tension and to its wetting properties upon the flexible, permeable covering of the organisms.

In studies on the influence of wetting agents on various antiseptics, Fisher[155] observed the enhancing effects of surface tension reducing agents upon mercuric chloride, mercury oxycyanide, chloramine T, argyrol and potassium permanganate. Little if any increase in germicidal activity was noted when the nonionic, anionic, or cationic surface tension depressants were added to phenol, cresol, or tincture of iodine.

Klarmann and Wright[156,157,158] attributed the irregular results obtained in the phenol coefficient tests on quaternary ammonium germicides to the powerful surface depressing action of the compounds. They considered the surface tension a function responsible for the "wild pluses" and possibly high phenol coefficient values that have been reported on this group of germicides. These factors were believed to be due to an unequal distribution of the test organisms in the medication mixture and also to the smaller volume of medication mixture carried over by the loop transfer to the subculture tube. In the latter respect, Tobie and Orr[159,160] reported a similar condition existing in the tests made on anionic surface-active compounds. They expressed their surprise that the diminution in volume of the test suspension, which was directly responsible to the low surface tension of anionic detergents, had not been considered by other investigators on surface-active materials. The relationship of surface-activity and the phenol coefficient test will be the subject of another section in this report.

DETERGENT ACTION

Mention was made in a previous section of this monograph that the quaternary ammonium germicides have been designated as "cationic wetting agents" or "cationic detergents." Both of these properties may be invested in a single compound. However, in general, while the quaternary ammonium germicides marketed today will materially lower the surface tension of water and are thus good "wetting agents," most of them have poor detergent properties.

Reference has already been made of the observations of Hornung,[144] Taub, *et al.*,[56,57,58] who noted the foaming action of the quaternary ammonium type of compounds. Bruson[161] described a number of organic nitrogen bases and salts that, although not mentioned as antibacterial agents, were believed to have excellent wetting, emulsifying, dispersing, and cleaning properties. The foaming action of tetrazolium salts has also been mentioned by Kuhn and Jerchel.[162] Epstein and his co-workers[32] compared the detergent action of a homologous series of fatty acid esters of colaminoformylmethylpyridinium chlorides. They noted from their studies that the C_{12}, C_{14}, and C_{16} derivatives were superior in detergent action to soap and about twice as active as other quaternary ammonium compounds which do not have carboxyl and amide linkages in the molecule.[163] They were unable to demonstrate any correlation between detergency and bactericidal action. The lauric acid and myristic acid ester derivatives described by Epstein were subsequently found by Penniston and Hedrick[164,165,166] to be effective detergents and germicides for washing dirty poultry eggs.

The use of relatively high concentrations of quaternary ammonium solutions, instead of soap, for cleaning unwashed skin and accidental wounds, will be the subject of another section of this review (page 147). Combinations of alkaline detergents and nonionic surface-active agents with quaternary ammonium germicides will also be discussed later.

STABILITY

An early reference to the stability and action of a quaternary ammonium compound upon bacteria and spores may be found in a publication by Thomann and Gschwind.[167] These investigators observed that a 0.1% solution of alkyldimethylbenzylammonium chloride would destroy a spore-free culture of bacteria within one hour while the same concentration failed to give a 100% destruction of bacterial spores in two hours at room temperature. However, by simultaneously heating the spore suspension and 0.1% quaternary ammonium solution at 100°C. an effective destruction of the spores was obtained in 30 minutes.

Kayser[168] advocated a preliminary scrubbing in 2% soda before boiling spore-contaminated instruments in solutions of alkyldimethylbenzylammonium chloride. He observed that of 100 contaminated instruments first scrubbed in soda and then boiled for 15 minutes in a 0.5% solution of the quaternary ammonium compound, only five were found to be contaminated. Upon boiling the same number of instruments in a 1% solution of the germicide only one was found contaminated. Treatment in 2% and 5% solutions of the compound resulted in 100% of the instruments becoming sterile.

Sabalitschka and Maas[169] determined the effects of alkyldimethylbenzylammonium chlorides upon bacterial spores at several pH values and under the influence of heat (100°C). Replicate tests at pH 5.8, 6.8, and 7.0 failed to show complete destruction of spores after 15 minutes of heating in the presence of the germicide. However, heating the suspensions for an additional 15 minutes resulted in a complete kill of the organisms. Survival of heat-treated spores after 30 minutes, in the absence of the quaternary ammonium compounds, was noted even when the pH of the solutions was reduced to 3.0. In the absence of the germicide, as well as an acid or base, the spores survived heating at 100°C. for three hours. Furthermore, heating for four hours resulted in sterility in only one instance out of seven cases.

Heineman[170] found that dilute solutions of alkyldimethylbenzylammonium chloride showed no decrease in phenol coefficients even when tested 14 months after their initial preparation. A 10% aqueous stock solution of the same quaternary ammonium germicide has been stored at room temperature in the writer's laboratories for at least 10 years with no evidence of a physical, chemical, or bacteriological change in properties. Furthermore, it has been observed that dilute aqueous solutions of the compound will readily withstand autoclaving at 20 pounds (126°C.) for at least an hour without any noticeable change in bactericidal efficiency.

Valko and DuBois[171] also found that a stock solution of alkyldimethylbenzylammonium chloride showed no change in germicidal activity which could be ascribed to aging after storage for over a period of at least one year. It is true that certain variations in the phenol coefficient results were obtained at different time intervals of testing the solution. However, these variations were considered inherent in the test procedure (F.D.A.[1] phenol coefficient) used for evaluating the quaternary ammonium compounds. A consideration of the phenol coefficient testing of these germicides will be made in a later section of this review.

Of the 40 surface-active agents, representing 12 classes of chemical compounds, Mueller, Bennett, and Fuller[94] found the quaternary ammonium and phosphonium compounds to be bacteriologically stable upon storage at room temperature for a period as long as two and one-half years. The hydrogen ion concentrations of these compounds were also shown to have no correlation with germicidal activity and stability of the surface-active agents.

Since some of the quaternary ammonium germicides available on the market today require the presence of isopropyl or related alcohols to maintain the compounds in concentrated solution, excess heat may drive off the organic solvent and thereby lead to the formation of a certain amount of insoluble salt in the aqueous solution. Mere dilution with a small volume of water in the absence of heat may also cause the formation of a precipitate in some of the less soluble quaternary ammonium germicides. Consequently, it is desirable to know the chemical constituents in the germicidal solution before an attempt is made to subject the material to heat stability tests.

True aqueous solutions of quaternary ammonium compounds will

[1] Food and Drug Administration.

withstand cold temperatures without change in the chemical constituent of the preparation. Precipitation or flocculation of the compound may occur, or the solution may even freeze at the lower temperature range, yet a clear solution will again result upon reheating the test samples. No chemical or bacteriological changes have been noted in solutions of alkyldimethylbenzylammonium chloride that have been subjected to freezing and then reconstituted to solution form by heat (Dunn,[172] Lawrence[173]).

It is apparent from the observations given that certain quaternary ammonium solutions will retain their germicidal activity when maintained for considerable lengths of time under normal storage conditions, and are, furthermore, not adversely affected by unreasonably cold or hot temperatures.

Quaternary ammonium germicides are nonvolatile and purified grades will present little if any odor. When the latter property is in evidence, the odor is slightly aromatic and altogether not objectionable. This attribute favors the use of the compounds in food processing plants, where they can be used on food utensils, milking and pasteurizing equipment, and on the food in process itself. This immediately precludes the use of phenols, cresols, pine oils, dyes, and related odoriferous substances.

6. Biology

PHENOL COEFFICIENT

While there have been many methods described for the determination of the bactericidal efficiency of a compound, the phenol coefficient test[174] is still used in the majority of laboratories for evaluating germicides and disinfectants. Most investigators will agree that the phenol coefficient values, even on phenolic disinfectants, may vary somewhat from day to day even when the test is carried out by the same individual. In comparing compounds unrelated to phenol, which kill bacteria by entirely different mechanisms, phenol coefficient methods may give varying or erroneous results. Nevertheless, the large number of compounds for which germicidal action is claimed by the manufacturer make it necessary that some yardstick for evaluation of disinfectants be established and recognized.

The anomalies that occasionally occur in the phenol coefficient results on highly active quaternary ammonium germicides has been a subject of considerable interest to many investigators during the past several years. These variations have been attributed to one or more factors related to the compounds or to the test procedure itself. Inconstancy of the composition of the culture media, particularly the peptones, has been considered responsible for some of the inconsistent end-points of phenol coefficient results on quaternary ammonium and related germicides.

Brewer[175] considered the phospholipid content of peptones an important factor in the germicidal testing of disinfectants containing wetting agents. He noted, moreover, that not all phospholipid substances would affect different synthetic detergents alike. For example, data was presented to show that lecithin was much more active than brain-heart medium in reducing the activity of alkyldimethylbenzylammonium chloride. When tested by the conventional phenol coefficient test, the latter germicide killed one strain of *E. typhosa* in a dilution of 1:30,000 and another strain at 1:20,000. By using 0.25% brain-heart medium in the test, both strains were destroyed in a dilution of 1:20,000. However, the presence of 0.1% lecithin in the medium prevented a 1:2,000 solution of the germicide from killing a test culture in 5 minutes. The interfering action of phospholipids (lecithin, cephalin, sphingomyelin) on the germicidal action of quaternary ammonium compounds was described earlier by Baker, Harrison and Miller[79] and more recently by Quisno, Gibby and Foter,[176] Stuart,[177] and Armbruster and Ridenour.[178] An account of these studies will be the subject of another section of this review.

To obviate inconsistent end-points in the phenol coefficient tests, semisynthetic media composed of more reproducible ingredients have been proposed by Wolf,[179] and Klarmann and Wright.[180] Both media contain acid hydrolyzed casein in place of peptone as the main source of nitrogen. When cultures of *E. typhosa* and *S. aureus* were grown in the new media, the authors noted that resistance to phenol was maintained for a longer period than when grown in F.D.A. medium. More recently Grubb and Edwards[181] found the phenol resistance of certain strains of *S. aureus* could be restored and maintained in F.D.A. broth merely by incubating the test culture at 40°C. instead of 37°C.

Mutation or "bacterial dissociation" of the test organisms, which may result in an increase or a decrease in sensitiveness of the bacteria to test solutions, has also been considered a contributing factor to fluctuations in end titers in the phenol coefficient test on quaternary ammonium germicides. Maier and Muller[182] early emphasized this point in their studies on the Rideal-Walker and F.D.A.[174] phenol coefficient testing of alkyldimethylbenzylammonium chloride.

A point which may bear consideration in this respect is the suggestion of Valko and DuBois[171] that a standard other than phenol be used for testing quaternary ammonium compounds. These investigators proposed the use of a reproducible, well-defined, crystalline quaternary ammonium germicide as a reference standard in the phenol coefficient or "quaternary ammonium coefficient" testing of this group of compounds.

The variations that occasionally occur in the phenol coefficient tests on highly active quaternary ammonium germicides have been attributed, in part, to an unequal distribution of bacterial cells in the test medium. Klarmann and Wright[156,158] reported that, in the presence of quaternary ammonium surface-active solutions, the organisms have a tendency to adhere to solid surfaces, i.e., walls of glass test tubes, thereby preventing a representative sample from being transferred to the subculture tube. However, substitution of rubber, plastic, or paraffin-coated test tubes in our tests, as well as in others, fails to show any significant differences in phenol coefficient values from those obtained in glass tubes.

Klarmann and Wright[156,157,158] also considered that an appreciable source of error in the phenol coefficient testing of quaternary ammonium germicides occurred in the loop transfer of the subcultures to broth medium. It was assumed that since the germicides were powerful surface tension depressants a smaller volume of fluid was carried over in the transfer as compared to the large volume transferred in tests on cresol, phenol, pine oil, and coal tar disinfectants. An analogous situation appears to have been noted in studies on anionic substances by Tobie and Orr.[159,160] Tensiometric surface tension measurements carried out by the writer reveal, however, that cresylic acid disinfectants with low phenol coefficients yield surface tensions comparable to or lower than those of many of the quaternary ammonium germicides having considerably higher phenol coefficient values. Table 11 presents a comparison of the surface tensions obtained on alkyldimethylbenzylammonium chloride, Lysol, and cresylic acid which demonstrates this point.

To obviate the possible inconsistency occurring in the volume of test mixture transferred by the loop method, Tobie and Orr proposed the use of a Kahn pipet for transferring the medication suspension. In this manner, exactly 0.02 ml., representing the volume carried by the standard 4-mm. loop, was used in testing surface-active solutions by the phenol coefficient test. By using this modification in the procedure they noted certain surface tension depressants did not potentiate the activity of germicides to quite as great a degree as did the loop transfer method.

Tice and Pressman,[101] on the other hand, noted that, while somewhat better results were obtained by the pipet transfer in the phenol coefficient testing of quaternary ammonium germicides, the method still gave results that were rarely reproducible. In their studies on the compatibility of quaternary ammonium antiseptics with positive and negative gelatins, 0.05 ml. amounts of inoculum were transferred to the subculture medium.

TABLE 11

SURFACE TENSIONS OF A QUATERNARY AMMONIUM GERMICIDE, LYSOL, AND CRESYLIC ACID*

DILUTION	QUATERNARY†	LYSOL	CRESYLIC ACID
1:10	34.0	33.7	31.0
1:20	34.9	33.7	31.5
1:40	35.6	34.0	31.8
1:60	35.8	33.5	32.0
1:80	36.0	32.6	31.7
1:100	36.0	31.6	31.0
1:200	36.2	28.2	31.0
1:400	35.0	28.1	29.1
1:600	35.0	29.1	28.0
1:800	35.1	31.0	28.1
1:1,000	34.5	33.1	30.5
1:2,000	35.2	36.0	33.9
1:4,000	39.1	39.6	39.2
1:6,000	43.0	42.2	39.4
1:8,000	46.0	46.3	40.3
1:10,000	46.5	49.1	43.0
1:20,000	53.8	59.1	46.5
1:40,000	60.2	59.4	47.3
Distilled water	(72.4 at 26°C.)		

* Determinations made with a Du Nuoy tensiometer, expressed in dynes per cm.

† Alkyldimethylbenzylammonium chloride.

In a further study to determine the sources of the apparent errors that occur upon testing the germicidal activity of quaternary ammonium compounds, Pressman and Rhodes[183] considered other factors which may be responsible for the discrepancy in results obtained in the phenol coefficient testing of these compounds. They changed the order of mixing by first adding 0.5 ml. of the cultures carefully to the bottom of the tube and then introduced the dilution of the germicide by allowing it to run down the side of the tube while being rotated. The medication tubes were not shaken at this point as required in the phenol coefficient procedure. In following the latter method, foaming was avoided, a factor Pressman and Rhodes considered a disadvantage in attempts to obtain reproducible results in testing quaternary ammonium germicides. Immediately at the end of the test period an aqueous, sterile solution of sodium stearate was added to the medication mixture to check further action of the quaternary ammonium compound upon the test organism. After mixing, 1 ml. of the suspension was plated and incubated at 37°C. for 48 hours. Using *E. typhosa* in the procedure outlined these investigators found that strikingly uniform results could be otbained by the pipet transfer method.

False end-points, due to "carry-over" of bacteriostatic amounts of quaternary ammonium germicides, have also been considered the cause of high values obtained in the phenol coefficient test. To differentiate between bacteriostatic and bactericidal activity, various agents have been added to the subculture medium in which transfers of the test suspensions of bacteria and quaternary ammonium compounds are made. While phenol coefficient tests were early made by Domagk[20] to determine the germicidal action of the cationic compounds in the presence of organic matter (in medication tube), it is only recently that attention has been directed to the addition of inactivating agents in the subculture broth (Quisno *et al.*,[176] Stuart,[177] and Armbruster and Ridenour[178]). A consideration of the bacteriostatic versus bactericidal aspect of quaternary ammonium germicides will be given later in this review.

By markedly modifying the regular F.D.A. phenol coefficient method for testing germicides, Klarmann and Wright[156,157] have attempted to demonstrate that this procedure is not applicable for the testing of quaternary ammonium compounds. One of the modifications involves the use of minute quantities of germicide (0.5 ml.) and test organisms (0.05 ml.) to which is added a fairly large volume of broth (20 ml. to 200 ml.) at the designated time intervals of 5, 10, and 15 minutes. The tubes or flasks are then incubated at 37°C. for 48 hours. They call attention, however, to a "pitfall" in this technic. This refers to the possible uneven distribution of the small volumes of test organisms and germicide solution which may result in untreated viable organisms in the culture medium which would develop to give a "false positive" test. Furthermore, in some instances, even in the largest volume of broth medium (200 ml.) they were able to demonstrate a bacteriostatic action of the quaternary ammonium germicide which inhibited the growth of the test organisms.

Another modification in the phenol coefficient test used by Klarmann and Wright consisted of using small volumes of germicide and test organisms to which was added melted and cooled agar (45°C.). After thorough mixing, the contents were poured into petri dishes and upon cooling were incubated at 37°C. The numbers of colonies developing in the plates were compared with the numbers treated with phenol in a corresponding manner. Two additional modifications in the test procedure involving agar plate counts of treated bacterial suspension were studied. These tests, which may be described as "Use Dilution" tests, will be discussed in the section bearing this title.

While the present writer and others do not question the authenticity of the results obtained by Klarmann[156] in the "broth dilution" method of phenol coefficient testing of quaternary ammonium germicides, there is a discrepancy in order of presentation of the results which may confuse the casual reader in evaluating the findings. The data consistently show the minimal concentrations and the phenol coefficient values of phenol, cresylic, phenolic, pine oil, and coal tar disinfectants that are germicidal to the test organisms as based upon the 100% pure substances. Yet in considering the quaternary ammonium germicides, the concentrations and phenol coefficient values are expressed in terms of the 10% or 0.1% concentrations of these compounds instead of 100% substances as before, thus making them appear fictitiously weak.

Klarmann and Wright[158] furthermore advanced the hypothesis that bacteria treated with quaternary ammonium germicides were agglutinated and the electrical charge imparted on them by the cationic agents caused the bacterial clumps to adhere to glass (test tubes), metal, or paper surfaces. This phenomenon, which is apparently lacking when organisms are treated with anionic surface-active agents, has been considered responsible for the coarse and irregular distribution of bacteria in solutions, resulting in erratic data obtained in the testing of quaternary ammonium germicides by the phenol coefficient technic.

The hypothesis advanced by Klarmann influenced the studies of McCulloch[17,115,184,185] who evaluated the quaternary ammonium germicides by plate counts as well as by use of the F.D.A. technics. This investigator assumed that the very rapid initial decrease in plate count numbers was a reflection of an agglomeration of the exposed bacteria and their migration and adherence to the walls of test tubes instead of an actual killing effect by the quaternary salts. McCulloch postulated, moreover, that the bacteria adhere to the transfer loop, and remain coated in the transfer medium with the cationic germicide, which results in their inability to multiply and carry on the normal functions of life, thereby creating bacteriostasis.

Vigorous responses to the attack by Klarmann on the adequacy of the F.D.A. phenol coefficient test in evaluating the quaternary ammonium germicides were made in many proposed modifications in the tests published after his reports. To mention a few, investigators proposing modified test procedures were Cade,[186,187] Klimek and Umbreit,[188] James and Eckfeldt,[189,190] Quisno, Foter, and Rubenkoenig,[191,192] and Barber *et al.*[121,193]

Cade considered the phenol coefficient method reliable for determining the germicidal potency of any and all disinfectants provided that certain modification be made in conducting the test. In collaboration with Halvorson,[194,195,196] they earlier proposed plate counts instead of broth subcultures for determining the presence or absence of viable organisms in the medication tubes at the designated time periods of exposure. All other features of the F.D.A. test were the same as originally described. Cade proposed, moreover, that the sides of the medication tubes be thoroughly swabbed with a sterile cotton applicator before an aliquot sample is plated in the agar medium. By using the modified Cade-Halvorson test procedure the "swab" method was found to parallel the efficiency of the "semimicro" technic of Klarmann and Wright.

Klimek and Umbreit were unable to confirm the work of Klarmann and Wright who reported that the high phenol coefficient values obtained with the quaternary ammonium germicides are "fallacious" and due to massive absorption of the organisms on the walls of the medication tubes. Klimek and Umbreit were able to show that analogous quantitative and qualitative variations may be obtained in determining the germicidal activity of phenol and alkyldimethylbenzylammonium chloride by the phenol coefficient test procedure. These variations in the test have been attributed primarily to the relatively small numbers of survival bacteria and to the statistical chances of including at least one or more of the viable organisms in the subculture sample.

Klimek and Umbreit were also able to account for the few surviving organisms after a relatively short exposure to the quaternary ammonium germicide. These viable organisms were present in the test suspension due to variations in the susceptibility of the individuals comprising the population of the inoculum. These investigators concluded that an evaluation of any germicide should require a quantitative test in which the number of organisms in the inoculum are known, and numbers of the surviving organisms are determined, rather than a test which gives merely qualitative information.

James and Eckfeldt combined the disinfectant and culture as described in the F.D.A. procedure but at the termination of the exposure they added a soap solution (sodium oleate) to the test mixture in order to prevent further action of the quaternary ammonium germicide on the test organisms. The walls of the medication tubes were swabbed as indicated in the Cade procedure to remove the adhering bacteria and 1 ml. of the dilution was plated in agar.

Quisno, Foter, and Rubenkoenig reviewed the methods that have been described for the evaluation of quaternary ammonium germicides and proposed a procedure of their own which they found would give reasonably reliable results. These investigators first added the culture to the medication tube and then the quaternary ammonium solution. The entire volume of 1 ml. was then placed in 10 ml. of broth containing a quaternary inactivating agent. In addition to the fluid medium the use of agar plates containing an inactivator was also proposed by these workers.

Barber and his co-workers used an "oval tube" method for determining the bactericidal effectiveness of various sterilizing solutions. This procedure consisted of inoculating the germicidal solution with a suspension of test organisms and transferring a loopful of the suspension, at specific time intervals, to oval tubes of melted agar. Following incubation at 37°C. the developing colonies are counted and the time required to obtain 99.9% destruction of the organisms is calculated.

Review of these publications reveals data and conclusions that are contradictory to the findings and hypotheses of Klarmann and his collaborators. In certain instances the opinions of the respective investigators have been sharply critical of Klarmann's views. Two of the more recent papers dealing with this controversy are those of DuBois,[197] and Klarmann and Wright[198] on the F.D.A. testing procedure for evaluating quaternary ammonium germicides.

The opposing points of view can be best expressed by direct quotations from the respective authors. Klarmann's[158] attitude is stated as follows: "The high phenol coefficient figures claimed for the quaternary ammonium compounds on a manifestly improper basis, also create an unjustified economic disadvantage to other germicides." Also in the same article, we note the following: "Reference is made to the economic disservice rendered to the position of other disinfectants by attributing to the quaternary ammonium compounds substantially greater germicidal powers than actually possessed by them."

In contrast, the stand of the majority of unbiased workers in this field is probably best expressed in a quotation from DuBois.[197] "There is an extensive literature presenting indirect, clinical or practical, evidence to show that the high molecular quaternary ammonium compounds are effective antiseptics and disinfectants. Disregarding this portion of our knowledge, there still remain data indicating that these compounds exert a definite bactericidal action."

Presentation of the several features that may be responsible for variations in phenol coefficient tests serves to indicate sources of errors that may be encountered in a single test procedure. At most, the laboratory or "test tube" results give no more than a suggestion or index of the actual value of the compound that is to be applied as a medicinal or general disinfectant. Nevertheless, having once established the relative merits of a specific antibacterial substance under actual conditions of use, there would appear to be no necessity for including a specification for the "clinical" or "practical" testing of the same compound. A simple phenol coefficient or "cationic coefficient" test for activity or chemical determination for the presence of the compound in solutions should suffice.

In regard to the chemical analysis of disinfectants, the chlorine industry alone, having once established the optimal concentration of germicidal activity of chlorine compounds, bears the distinction of buying and selling their products on a concentration basis. Chemical determination of "available chlorine" provides users and inspectors with a rapid method of checking the strength of the solution in use and for purchasing specifications.

The Disinfectant Scientific Committee of the National Association of Insecticide and Disinfectant Manufacturers, in direct cooperation with the United States Department of Agriculture, has recently completed a series of collaborative studies on bacteriological methods for testing quaternary ammonium germicides. A report on the results of these studies may be found in the publication by Stuart.[177] The Chemical Analysis Committee of the Association is, at present, actively investigating simplified and standardized chemical methods for the evaluation of the compounds.

At the present time, the quaternary ammonium manufacturer is guided by the United States Department of Agriculture which specifically states in Circular 198[174] the following: "All antiseptics and disinfectants shipped or offered for import into or export from the United States are subject to the provisions of the Federal Insecticide Act, the Federal Food and Drugs Act, or both. In the enforcement of these acts, it is necessary to determine the accuracy of the bactericidal and antiseptic claim made for such products. A number of methods have been developed for determining bactericidal effectiveness, but all of them possess certain disadvantages. Of course, it is impossible to devise tests which will apply in all cases, but during the past 20 years the Insecticide and Fungicide Board and the Food and Drug Administration have found certain methods to be particularly well adapted to their purposes."

Until such time as an approved modification of the phenol coefficient test is compulsory for testing quaternary ammonium compounds, the manufacturer of this group of germicides is justified in making label claims for phenol coefficients on his product. The general trend for such modifications is not along *in vitro* test methods but rather toward "Use Dilution" tests that more closely represent conditions under which the compounds will actually be used.

In concluding this section it appears appropriate to indicate the range of phenol coefficient values that may be expected in the routine testing of several commercially available quaternary ammonium germicides. In tabulating this information, the results are presented only for *S. aureus* and *E. typhosa,* using the data obtained in the conventional Food and Drug Administration (F.D.A.) or Rideal-Walker phenol coefficient procedure. Table 12 presents references and the results of a series of these tests. From the data given in the table it is evident that the quaternary ammonium germicides, in general, give high phenol values. Only an occasional phenolic,[38,150] or mercurial[199] compound has been reported to give the same order of degree in germicidal activity.

USE DILUTION

Certain investigators have proposed the use of a "carrier" other than the standard platinum loop for transferring a germicide-treated culture from the medication tube to the broth subculture medium. In recent years this method of testing has been designated as the "Use Dilution" technic.

Mallmann and Hanes,[205] and Mallmann and Leavitt[206] described a procedure that consists of dipping standard cylinders of glass in a culture of organisms, allowing the culture to dry on the glass, and then exposing the treated cylinders to a series of dilutions of the disinfectant. At varying time intervals, a treated cylinder is removed from the medication mixture and dropped directly in tubes of sterile nutrient medium. After thorough shaking, representative samples of the broth suspension are plated in agar. The number of colonies developing in the latter plates are counted and compared with the numbers growing in plates inoculated with untreated controls.

TABLE 12

PHENOL COEFFICIENTS ON QUATERNARY AMMONIUM GERMICIDES

QUATERNARY	AUTHOR	*S. aureus*		*E. typhosa*	
		20°	37°	20°	37°
Alkyldimethylbenzylammonium·Cl	Kliewe[200]	150	.	200	. . .
Alkyldimethylbenzylammonium·Cl	Dunn[22]	279	407	250	429
Alkyldimethylbenzylammonium·Cl	Heineman[170]	275	293	176	271
Alkyldimethylbenzylammonium·Cl	Deskowitz[199]	362	324	317	310
Alkyldimethylbenzylammonium·Cl	Maier[204]	150	. . .	200	. . .
Alkyldimethylbenzylammonium·Cl	Lawrence[201 202]	250	325	275	314
p-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium·Cl	Lawrence[203]	323	300	200	257
9-octadecenyldimethylethylammonium·Br	Lawrence[203]	250	262	133	228
Cetylpyridinium·Cl	Lawrence[203]	350	337	133	228
N-(acylcolaminoformylmethyl)pyridinium·Cl	Lawrence[203]	150	200	111	200
N-(acylcolaminoformylmethyl)pyridinium·Cl	Epstein[31]	500	571	222	343
Cetyltrimethylammonium Br·pH 5.0	Hoogerheide[147]	300	650	.	. .
Cetyltrimethylammonium Br·pH 6.7	Hoogerheide[147]	500	1,000	.	.

Reference has already been made to the theory advanced by Klarmann and Wright[156,157,158] relative to the migration of organisms to solid surfaces when in solutions of quaternary ammonium germicides. They attributed some of the discrepancies noted in the phenol coefficient testing of these compounds to this phenomenon. To demonstrate the tendency of bacteria to adhere to solid surfaces (i.e. glass, etc.), Klarmann and Wright used glass strips, instead of glass cylinders, for tranferring bacteria exposed to quaternary ammonium solutions. At the end of 10 minutes' exposure the glass strips are removed and plated in agar. At the same time, 0.05 ml. of the same test suspensions are removed with a pipet and also plated in agar. The latter volume of sample is considered to be the amount of material carried over by the glass strip. Although they were able to demonstrate that more colonies developed in the plate containing the glass strip than when the solution was transferred by means of a pipet, they failed to take under consideration the number of organisms that "adhered" to the inner part of the pipet and were, therefore, not plated.

In a consideration of Klarmann's results, Mallmann[207,208] used a "speed of reaction" test designed to prevent a sufficient amount of time for migration of the test organisms to the walls of test tubes. The study involved the addition of 1 ml. of test culture to 10 ml. of the quaternary ammonium dilution. One milliliter of the test suspension was removed at intervals of 5, 10, and 15 seconds and placed in 9 ml. of sterile saline containing soap as the quaternary "neutralizing" agent. Representative samples of this solution, or dilutions thereof, were then plated in agar. Mallmann demonstrated in these tests that a dilution of 1:20,000 alkyldimethylbenzylammonium chloride gave a complete kill for *S. aureus* and *Micrococcus caseolyticus* in 10 seconds. A 1:10,000 dilution was found to give a complete kill for the latter organisms as well as *E. coli* in 5 seconds.

Johns[209] used glass slides dipped in a skim-milk suspension of test organisms as a means of comparing the germicidal activity of hypochlorite and quaternary ammonium solutions. The milk suspension of organisms was allowed to dry partially on the slides following which the latter were immersed and gently agitated in the germicide solutions. They were then rinsed in sterile water and placed in a Petri dish, and melted and cooled agar was poured over the slides. When the medium had cooled, the plates were incubated at 37°C. Destruction of 99.9% of the organisms was considered to be an endpoint. It was noted that more reproducible results could be obtained by the procedure outlined than by the ordinary F.D.A. phenol coefficient test. In general, the quarternaries were found to be more effective against Gram-positive microorganisms (*S. aureus, M. candidus,* and spores of *Bacillus panis*) while the hypochlorites were "decidedly" superior against the Gram-negative bacteria (*E. coli* and *Ps. aeruginosa*).

Stuart[177] has recently described a third means of using glass "carriers" for transferring medicated culture suspensions to nutrient media. In this test, use is made of Pyrex glass rings which are first dipped in a broth culture of test organisms, allowed to dry at room temperature for 30 minutes and then exposed to the germicide dilution. At intervals of 5, 10, and 15 minutes one of three rings is removed from the medication tube by means of a platinum hook and transferred to the subculture medium. The latter medium may be plain F.D.A. broth, one containing serum as the quaternary inactivating agent, or a medium in which lecithin and Tween 80 are added as neutralizing agents (Quisno, Gibby, and Foter[176]). Table 13 presents the results of several series of phenol coefficient tests carried out in our laboratories in which the several procedures recommended by Stuart were used. It is obvious from the data given that by proper selection of laboratory technic, one can demonstrate almost at will that the quaternary ammonium germicides are either poor or unusually good germicides.

Brief mention may also be made of the observations of Klarmann[156] who found that a marked diminution in phenol coefficient values on quaternary ammonium compounds could be obtained by using the "filter paper" technic described in Circular 198 of the U. S. Department of Agriculture. Further considerations of the desirability for supplanting the F.D.A. phenol coefficient tests by "Use Dilution" methods for evaluating the quaternary ammonium germicides are presented in the publications by DuBois,[210] Reddish,[211] Bernstein, Epstein, and Wolk,[212] and others.

BACTERICIDAL TESTS

A review of the literature reveals that in determining the germicidal activity of quaternary ammonium compounds there are relatively few phenol coefficient values given in comparison to the "limiting" antibacterial dilutions on these compounds. While Jacobs and his co-workers described the synthesis of the large series of hexamethylenetetramine salts in 1915, mention was first made of studies on the bacteriology of these compounds in 1916.[18] In determining the antibacterial activity of their compounds, 0.5 or 1.0% solutions

TABLE 13

Comparison of "Use Dilution" and Phenol Coefficient (F.D.A.) Methods for Testing Quaternary Ammonium Germicides

		S. aureus				*E. typhosa*			
		Transfer Min.			Phenol	Transfer Min.			Phenol
Quaternary	Dilution	5	10	15	Coeff.	5	10	15	Coeff.
I. Glass rings used as "carriers" for subculture to a quaternary inactivating medium.*									
	1: 9,000	−	−	−		−	−	−	
	1:12,000	−	−	−		−	−	−	
	1:15,000	+	+	−		+	−	−	
"B"†	1:18,000	+	+	−	$\frac{14,000}{75}=187$	+	+	−	$\frac{15,000}{95}=158$
	1:21,000	+	+	+		+	+	−	
	1:24,000	+	+	+		+	+	−	
	1:27,000	+	+	+		+	+	+	
	1: 6,000	+	−	−		−	−	−	
	1: 9,000	−	−	−		+	−	−	
"C"†	1:12,000	+	+	−	$\frac{9,000}{70}=128$	+	+	+	$\frac{9,000}{95}=95$
	1:15,000	+	+	−		+	+	+	
	1:18,000	+	+	−		+	+	+	
	1:21,000	+	+	+		+	+	−	
	1: 6,000	−	−	−		−	−	−	
	1: 9,000	−	−	−		−	−	−	
"E"†	1:12,000	+	−	−	$\frac{15,000}{70}=214$	+	+	−	$\frac{11,000}{95}=115$
	1:15,000	+	−	−		+	+	−	
	1:18,000	+	+	+		+	+	−	
	1:21,000	+	+	+		+	+	+	
II. Standard 4-mm. loop used for subculture to a quaternary inactivating medium.*									
	1:12,000	−	−	−		−	−	−	
	1:15,000	+	−	−		−	−	−	
"B"	1:18,000	−	−	−	$\frac{21,000}{65}=323$	+	−	−	$\frac{21,000}{90}=233$
	1:21,000	+	−	−		+	−	−	
	1:24,000	+	+	−		+	+	−	
	1:27,000	+	+	+		+	+	+	
	1: 9,000	+	−	−		−	−	−	
	1:12,000	+	−	−		+	−	−	
"C"	1:15,000	+	−	−	$\frac{15,000}{65}=230$	+	+	−	$\frac{12,000}{90}=133$
	1:18,000	+	+	−		+	−	−	
	1:21,000	+	+	−		+	+	−	
	1:24,000	+	+	+		+	+	−	
	1: 9,000	−	−	−		−	−	−	
	1:12,000	+	−	−		−	−	−	
"E"	1:15,000	+	−	−	$\frac{15,000}{65}=230$	+	−	−	$\frac{15,000}{90}=167$
	1:18,000	+	+	−		+	+	+	
	1:21,000	+	+	−		+	+	+	

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* = Modified Letheen Broth.

† "B" = Alkyldimethylbenzylammonium chloride. "C" = Cetylpyridinium chloride.
"E" = *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride.

were prepared in distilled water and sterilized by Berkefeld filtration. The stock solution was serially diluted with distilled water and to 5 ml. quantities of the solution was added 0.1 ml. of an undiluted broth culture of test organism. At the end of 3 hours, a 4-mm. loopful of the suspension was plated in agar and a loopful also transferred to nutrient broth. The numbers of colonies developing in the agar plates and the first broth tube in the dilution series showing no growth were recorded at the end of 48 hours' incubation at 37°C.

Jacobs and his co-workers[14] early abandoned the use of the agar plate in preference to broth medium in testing the hexamethylenetetramine derivatives. This was based on their observations that an abrupt decrease in the number of colonies was noted at the same or almost the same dilution indicated by no growth in the broth tubes. Also, while marked turbidity would be noted in one concentration, the next or higher concentration in the series would remain absolutely clear. Their results, furthermore, revealed that from 50 to 80 viable organisms were necessary to elicit visible growth in 10 ml. of broth within 48 hours. In the agar plate, on the other hand, each organism produced a colony within the same incubation period. In comparing several of the salts of hexamethylenetetramine (chloroacetamide, chloroacetylmethylamide, chloroacetyldimethylamide, etc.) and several commonly used organic antiseptics of that time, dilutions of 1:1,600 of the quaternary ammonium compounds killed *E. typhosa* in 3 hours at 20°C. In the same test, formaldehyde gave the same effect in a dilution of 1:1,200; Lysol 1:400; trichlorophenol 1:800; and tribromo-*p*-cresol in a dilution of 1:1,600. A 1:200 dilution of phenol failed to give a complete kill of *E. typhosa* in this study.

Domagk's[21] early reference to a dilution of 1:50,000 of a quaternary ammonium compound that would kill *E. coli* and *S. aureus* in 10 and 15 minutes, respectively, failed to mention the test method used in the derivation of this value.

Table 14 presents a number of references to studies in which the limiting killing dilutions of various quaternary ammonium germicides were determined. In all instances the organisms were added to aqueous solutions of the compounds and subcultures made to nutrient broth as outlined in the F.D.A. or Rideal-Walker methods for phenol coefficient testing. In summarizing the data given in the table, it will be noted that dilutions of 1:16,000 to 1:67,000 of several of the quaternary ammonium compounds listed will destroy *S. aureus* within 10 minutes at 20°C. Considerably higher dilutions are effective when testing the compounds at 37°C. With but few

TABLE 14

LIMITING DILUTIONS OF QUATERNARY AMMONIUM COMPOUNDS EFFECTIVELY KILLING ORGANISMS AT SHORT TIME INTERVALS OF EXPOSURE

QUATERNARY	AUTHOR	KILLING TIME	*S. aureus*		*E. typhosa*		*E. coli*	
			20°	37°	20°	37°	20°	37°
Alkyldimethylbenzylammonium·Cl	Schneider[87]	1 min.		16,000				7,000
Alkyldimethylbenzylammonium·Cl	Deskowitz[199]	10 min.		30,500		45,300		
Alkyldimethylbenzylammonium·Cl	Dunn[172]	10 min.	20,000		20,000		12,000	
Alkyldimethylbenzylammonium·Cl	Lawrence[201,202]	10 min.	18,000		27,000		27,000	
Cetylpyridinium·I	Kolloff[29]	10 min.		50,000				
Cetylpyridinium·Cl	Quisno[213]	10 min.	67,500	110,000	62,500	83,500		66,000
Cetyltrimethylammonium·Br	Hoogerheide[117]	10 min.	34,000	80,000				
9-octadecenyldimethylbenzylammonium·Cl	Valko[34]	10 min.	29,000	33,000	10,000			
Dodecyldimethylbenzylammonium·Cl	Valko[34]	10 min.	20,000		16,000			
Hexadecyldimethylbenzylammonium·Cl	Valko[34]	10 min.	14,000	22,000	11,000			
Hexadecylpyridinium·Br	Valko[34]	10 min.	25,000		5,000			
***N*-(lauric acid ester of colaminoformylmethyl) pyridinium·Cl**	Epstein[31]	10 min.	14,000	25,000	10,000	30,000		
***N*-(myristic acid ester of colaminoformylmethyl)pyridinium·Cl**	Epstein[31]	10 min.	30,000	55,000	30,000	55,000		

TABLE 14—(*Continued*)

LIMITING DILUTIONS OF QUATERNARY AMMONIUM COMPOUNDS EFFECTIVELY KILLING ORGANISMS AT SHORT TIME INTERVALS OF EXPOSURE

QUATERNARY	AUTHOR	KILLING TIME	*S. aureus*		*E. typhosa*		*E. coli*	
			20°	37°	20°	37°	20°	37°
p-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium·Cl	Rawlins[33]	5 min.	25,000	......	12,000	......	......	......
p-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium·Cl	Joslyn[85]	5 min.	25,000	50,000	12,000	20,000	......	......
***N*-diethyl-*N*-benzyl-*N*-(*p*-cetylaminophenoxyethyl)ammonium·Cl**	Taub[57]	2½ min.	1,000	.. .	.. .	. ...	250	.. ...
***N*-diethyl-*N*-benzyl-*N*-(*n*-octyloxyphenthioethyl)ammonium·Cl**	Taub[57]	2½ min.	25,000	.. . .	.. . .	....	10,000	......
***N*-diethyl-*N*-benzyl-*N*-(3-butylamino-4-cresyloxyethyl)ammonium·Cl**	Taub[57]	2½ min.	50,000	.	.. ..	......	5,000	......
3-, 6- and 8-oxyquinolinedodecylethermethosulfates	Kuhn[45]	10 min.	...	.. ...	.....	... .	12,000	......
2, 3-diphenyl-5-n-undecyltetrazolium·Br	Kuhn[162]	10 min.	38,400	..	.. .	.. .	2,400	.... .
Diphenylundecyltetrazolium·Cl	Jerchel[50]	10 min.	38,400	......	.. ..	......	. ....	......

exceptions, the quaternary ammonium germicides exhibit a slight to marked diminution in activity when tested against the Gram-negative bacilli, *E. typhosa* and *E. coli,* as compared to their action against the Gram-positive coccus, *S. aureus.*

Using the procedure outlined in Circular 198 of the U. S. Department of Agriculture, Quisno and Foter[213] determined the average "critical killing dilution"[j] of cetylpyridinium chloride against a variety of pathogenic bacteria. A reproduction of their table presenting the bactericidal spectrum of the quaternary ammonium germicide is given in Table 15.

TABLE 15

GERMICIDAL ACTIVITY OF CETYL PYRIDINIUM CHLORIDE AQUEOUS SOLUTION (Quisno and Foter, *Jour. Bact.*, 52, 111, 1946)

ORGANISM	NO. STRAINS TESTED	AVERAGE CRITICAL KILLING DILUTION IN TERMS OF ACTIVE INGREDIENTS AT 37°C.	
		NO SERUM	10% BOVINE SERUM
Staphylococcus aureus	5	1:83,000	1:12,500
Staphylococcus albus	1	1:73,000	1:12,000
Streptococcus viridans	1	1:42,500	1:12,000
Streptococcus hemolyticus	2	1:127,500	1:17,000
Neisseria catarrhalis	2	1:84,000	1:13,000
Diplococcus pneumoniae I	1	1:95,000	1:14,000
Diplococcus pneumoniae III	1	....	1:20,000
Pseudomonas aeruginosa	2	1:5,800	1:1,000
Klebsiella pneumoniae	2	1:49,000	1:5,500
Corynebacterium diphtheria	1	1:64,000	1:14,000
Mycobacterium phlei	1	1:1,500	1:1,000
Eberthella typhosa	5	1:48,000	1:3,000
Escherichia coli	2	1:66,000	1:1,000
Proteus vulgaris	2	1:34,000	1:2,000
Shigella dysenteriae	1	1:60,000	1:5,000
Shigella paradysenteriae (Flexner)	2	1:52,000	1:3,500
Shigella paradysenteriae (Hiss)	1	1:49,000	1:2,000
Shigella sonnei	2	1:68,000	1:6,500
Lactobacillus acidophilus	1	....	1:16,500
Brucella abortus	1	....	1:19,500
Trichomonas vaginalis	1	....	1:3,000*
Candida albicans	1	1:37,000	1:3,500
Cryptococcus neoformans	1	1:61,000	1:6,000
Trichophyton mentagrophytes	1	1:36,000	1:3,000
Microsporum canis	1	1:34,000	1:5,000

* Twenty-five per cent human serum.

When allowed to act upon bacteria for periods longer than those encountered in the phenol coefficient tests, considerably higher dilutions may be expected to destroy microorganisms. In carrying out these studies, the routine test procedure usually involves the addition of quaternary ammonium dilutions to nutrient broth to which are added the test organisms. The tubes are incubated at 37°C. for several days and presence or absence of visible growth noted. To distinguish between bactericidal and bacteriostatic effects, subcultures may be made from the incubated test solutions to fresh tubes of broth or agar plates. The latter media are also incubated at 37°C. and observed for the appearance or absence of growth. Table 16 presents the results of limiting dilutions of a number of quaternary ammonium compounds that have been reported to be bactericidal and/or bacteriostatic to *S. aureus*. Examination of the data again reveals the high germicidal and inhibiting properties of the quaternary ammonium compounds against the Gram-positive cultures of *S. aureus*.

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[j] Highest dilution killing in 10 but not in 5 minutes.

TABLE 16

LIMITING DILUTIONS OF QUATERNARY AMMONIUM COMPOUNDS EFFECTIVELY KILLING OR INHIBITING GROWTH OF *S. AUREUS* IN BROTH MEDIUM

QUATERNARY	AUTHOR	*S. aureus* Bc*	Bs*
o-trimethylammoniumphenol-*n*-dodecylether methosulfate	Kuhn[44]	4,800	19,200
m-trimethylammoniumphenol-*n*-dodecylether methosulfate	Kuhn[44]	4,800	19,200
p-trimethylammoniumphenol-*n*-dodecylether methosulfate	Kuhn[44]	1,600	25,600
Dodecyldimethylbenzyl-ammonium • Br	Kuhn[44]	1,200	12,800
Alkyldimethylbenzylammonium • Cl	Jerchel[50]	38,400	200,000
Diphenylundecyltetrazolium • Cl	Schneider[87]	......	409,600
2-phenyl-3-α-naphthyl-5-*n*-undecyltetrazolium • Br	Kuhn[162]	38,400	76,800
2-phenyl-3-*n*-bromphenyl-5-*n*-undecyltetrazolium • Br	Kuhn[162]	2,400	4,800
Cetyltrimethylammonium • Br	Williams[214]	218,700	.....
Cetylpyridinium • Br	Williams[214]	218,700	.....
Alkyldimethylbenzylammonium • Cl	Deskowitz[199]	......	320,000
Alkyldimethylbenzylammonium • Cl	Lawrence[201,202]	200,000	800,000
Decyltrimethylammonium • Br	Hoogerheide[147]	......	7,000
Dodecyltrimethylammonium • Br	Hoogerheide[147]	......	70,000
Tetradecyltrimethylammonium • Br	Hoogerheide[147]	......	340,000
Cetyltrimethylammonium • Br	Hoogerheide[147]	......	400,000

* Bc = Bactericidal. Bs = Bacteriostatic.

In order to determine the bactericidal and bacteriostatic spectra that may be obtained on homologous series of quaternary ammonium compounds, Lawrence, Kwartler, Wilson, and Kivela[49] tested several derivatives of alkyldimethylbenzylammonium chlorides against a variety of pathogenic and some nonpathogenic bacteria. Stock (1%) solutions of the compounds were prepared in distilled water. Serial dilutions were made from the stock directly in broth most suited for the organisms to be tested. The drug-broth solutions were distributed in 10 ml. quantities in test tubes and autoclaved at 10 pounds for 10 minutes. The inoculum, in most instances, consisted of 0.2 ml. of a 1:1,000 dilution of a 24-hour broth-diluted culture of test organism. A number of Gram-negative bacilli representative of the colon-typhoid-dysentery group was tested in the presence of F.D.A. broth. The latter medium was also used for *Proteus, Pseudomonas,* and *Vibrio* (Cholera) cultures. Veal-dextrose broth containing 0.1% normal horse serum was used in tests in which *S. aureus,* Type I, II, and III pneumococci, and *S. pyogenes* (#C-203) were studied. Brewer's fluid thioglycollate medium was selected for studying the effects of the quaternary ammonium compounds against the anaerobic clostridia. The inoculum used in the studies on the latter group of bacteria was a 4-mm. loopful of a 24-hour undiluted thioglycollate culture of test organism.

The inoculated media were incubated at 37°C. and observed for the presence of visible growth at the end of 24, 48, and 72 hours. Failure of growth to appear at the initial 24-hour incubation period was considered evidence of bacteriostasis. Tubes showing no growth at the end of 72 hours were tested for bactericidal action by transferring three 4-mm. loopfuls of the test mixture to a second tube of liquid medium. Lack of growth in the subculture tubes following 72-hour incubation was taken as evidence of a bactericidal action of the quaternary ammonium compound upon the test organisms in the original broth tube.

Table 17 presents typical results obtained upon using three representative compounds in two of the series of alkyldimethylbenzylammonium chlorides. In reviewing the data of the first three compounds (I, II, III), which represent the lorol,[k] cetyl, and octadecyl unsubstituted benzyl group, it will be noted that the first two compounds give comparable degrees of activity against the entire group of organisms. Concentrations of approximately 1:128,000 are lethal for the typhoid, dysentery, and cholera bacteria, whereas considerably greater concentrations are required to destroy the remaining Gram-negative organisms. The Gram-positive cocci and anaerobic clostridia are uniformly sensitive to the two derivatives in dilutions of 1:200,000 to 1:400,000.

[k] See footnote page 10.

TABLE 17

LIMITING DILUTIONS OF SEVERAL ALKYLDIMETHYLBENZYLAMMONIUM CHLORIDES SHOWING ANTIBACTERIAL EFFECTS IN NUTRIENT MEDIA

	COMPOUND-DILUTION*											
	I†		II		III		IV		V		VI	
Organism—Strain	Bs‡	Bc‡	Bs	Bc	Bs	Bc	Bs	Bc	Bs	Bc	Bs	Bc
E. typhosa "H"	256	128	256	128	16	8	128	128	128	128	16	16
S. dysenteriae "F"	512	256	128	32	32	8	256	128	256	128	16	8
E. coli	64	32	256	128	4	4	128	32	64	16	4	2
A. aerogenes	16	16	32	16	1	<1§	32	32	32	16	<1	<1
S. paratyphi	64	32	32	32	4	2	64	32	32	32	1	1
S. enteritidis	32	32	16	16	4	2	32	32	32	32	<1	<1
Proteus vulgaris	16	8	16	16	8	4	16	16	32	16	128	4
Ps. aeruginosa "I"	32	32	64	16	1	1	64	64	64	32	<1	<1
V. cholera "As"	512	256	256	128	64	16	256	64	256	128	4	4
S. aureus	800	200	400	200	50	50	200	100	800	100	50	25
Pneumo. II	200	200	800	400	800	400	800	400	800	200	400	400
S. pyogenes	800	200	1,600	400	800	400	800	400	1,600	400	800	400
Cl. welchii	200	200	400	400	25	25	800	800	200	200	50	50
Cl. tetani	200	200	400	200	100	25	800	800	400	400	100	50
Cl. histolyticum	200	50	400	400	100	25	800	800	800	200	50	25
Cl. oedematiens	200	200	200	200	25	25	800	800	50	50	50	50

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* All values given represent dilutions in thousands, i.e., 256 = 1:256,000.

† I = lorolbenzyl, II = cetylbenzyl, III = octadecylbenzyl, IV = dodecyl 3, 4-dichlorobenzyl, V = myristyl 3, 4-dichlorobenzyl, VI = cetyl 3, 4-dichlorobenzyl.

‡ Bs = compound-dilution bacteriostatic; Bc = compound-dilution bactericidal.

§ Figures preceded by < indicate the highest concentration of cationic compound tested and failed either to kill or inhibit the growth of the test organism.

Upon increasing the number of carbon atoms in the chain from C_{16} (II-cetyl) to C_{18} (III-octadecyl), an abrupt drop in activity occurs against all Gram-negative bacteria, Gram-positive clostridia and *S. aureus* cultures. The pneumococci and β-hemolytic streptococcus culture, on the other hand, are as sensitive to the octadecyl as they are to the cetyl or lorol compounds.

The data obtained on the second group of compounds (IV, V, VI), which represent the dodecyl (C_{12}), myristyl (C_{14}), and cetyl (C_{16}) derivatives of 3,4-dichlorobenzylammonium chloride, indicate that an abrupt drop in activity occurs when the carbon chain lengths are increased from C_{14} to C_{16}. The C_{12} and C_{14} derivatives give results comparable to those obtained with the lorol and cetyl derivatives of the unsubstituted alkyldimethylbenzylammonium chlorides (I, II). Furthermore, there is a close correlation in the degrees of activity of the octadecyl (C_{18}) derivative of the latter group of compounds and the cetyl analogue of 3,4-dichlorobenzylammonium chloride.

Nagel[215] described a test method for determining the germicidal activity of quaternary ammonium compounds in which the bacterial cells were washed after treatment with the sterilizing solution. The procedure involved the addition of 0.1 ml. of a heavy test suspension of bacteria to sterile centrifuge tubes to which was added 0.9 ml. of the desired concentration of the quaternary ammonium compound. At periods of 1 to 15 minutes, the organism-quaternary ammonium mixtures were centrifugated, the supernatant discarded, and the sedimented cells resuspended in saline. Following two washings and centrifugations the bacterial cells were streaked on blood-agar plates, as well as inoculated in fluid medium. Lack of visible growth to appear in the media following 48 hours' incubation at 37°C. was taken as evidence of a kill of the test organism by the quaternary ammonium compound. Although Nagel was not concerned in determining the limiting dilutions of a quaternary ammonium compound which would destroy his test organisms, data were presented to show that a 0.25% concentration would destroy staphylococci, streptococci, pneumococci, and coliform organisms within one minute treatment as evidenced by his test results. A spore-forming "hay bacillus" required concentrations of 1 to 2% for complete destruction in 15 minutes, whereas the tubercle bacillus was not killed by a 2% solution even after 24-hour exposure to the quaternary ammonium germicide.

The procedure of washing bacterial cells after treatment with a quaternary ammonium or other germicide has also been used by

Miller.[216] This investigator tested several salts of heavy metals, halogens, phenols, alcohols, quinones, and anionic and cationic compounds against virulent cultures of meningococci. The organisms were exposed to the germicides for 15 to 30 minutes and then centrifugated and washed several times with physiological saline solution. The final sediment of bacterial cells was streaked onto surfaces of blood-agar plates. In using this test procedure, Miller found the heavy metal compounds were most effective against the meningococci. Next, in order of activity, were quinone and hydroquinone, iodoacetic acid, and the cationic germicide, alkyldimethylbenzylammonium chloride. The latter killed the organisms regularly at 0.025% and usually at 0.005% (1:4,000-1:20,000).

Eisman and Mayer[217] more recently described a new quaternary ammonium germicide, phenoxyethyldimethyldodecylammonium bromide, with reported phenol coefficient values of 433 and 521 against *E. typhosa* and *S. aureus* respectively. Dilutions ranging between 1:24,000 and 1:768,000 were found to be bacteriostatic and fungistatic to a variety of organisms tested.

INFLUENCE OF pH ON ANTIBACTERIAL EFFICIENCY

In studies on the effects of alkyldimethylbenzylammonium chloride, Dunn[172] reported that the germicide exerts a greater bactericidal action at an alkaline pH than in the acid range. Miller and Baker[218,219] also noted that the pH of a buffer medium would cause striking differences in inhibitory action. It was found that the antibacterial effects of the cationic detergents (quaternary ammonium) increased progressively as the pH shifted toward the alkaline side. The anionic detergents, on the other hand, became more active when the shift was in the acid range.

Gershenfeld and Milanick,[145] and Gershenfeld and Perlstein[146] concluded from their studies that the hydrogen ion concentration was one of three important factors concerned in an environment which determines the bactericidal efficiency of surface tension depressants. It was implied, therefore, that reduced surface tension alone does not explain the bactericidal action of surface-active agents. Blubaugh, Botts, and Gerwe[220] studied the effects of changes in pH on the germicidal activity of cetylpyridinium chloride, but no data or references to the effect of lowering or raising the pH were given. An exception to the response of most quaternary ammonium germicides to changes in the pH of the medium are the results obtained by Quisno and Foter[213] on cetylpyridinium chloride.

These workers reported that the germicidal action of the latter compound was as effective at the extreme acid pH of 2.0 as it was at the alkaline range of 10.0. The reason for the marked antibacterial action of cetylpyridinium chloride in an acid environment as compared to the ineffectiveness of other quaternary ammonium germicides in the pH ranges of 4.0 or lower has not, as yet, been determined.

In a consideration of the factors that influence the germicidal action of alkyltrimethylammonium bromides, Hoogerheide[117] observed that the compounds are more active at pH 8.0 than at pH 5.0. However, at the latter pH some of the derivatives are still highly potent germicides. Reference has already been made to the studies of Sabalitschka and Maas[169] (page 52) who were unable to demonstrate complete destruction of bacterial spores by heating quaternary ammonium solutions at 100°C. for 15 minutes at pH 5.8, 6.8, or 7.0. However, a complete kill was obtained when the time of heating was increased to 30 minutes. In the absence of the germicide the spores survived heating at 100°C. even at a pH of 3.0.

The relation of the pH of alkyldimethylbenzylammonium chloride to germicidal activity against *S. aureus* was determined in our laboratories. To 100-ml. quantities of the germicide dilutions was added N/10 HCl or N/10 NaOH. The pH determinations were made with a glass-electrode potentiometer soon after the addition of the latter solutions and again after the adjusted solutions were allowed to stand at room temperature for several days. To 5 ml. quantities of test solution was added 0.1 ml. of a 24-hour culture of *S. aureus*. A 4-mm. loopful was transferred from the tube to F.D.A. broth at time intervals of 5, 10, and 15 minutes. The temperature at which the tests were carried out was 22-25°C. The tubes containing the transplants were incubated at 37°C. for 72 hours at which time the results were recorded.

From Table 18 the following will be noted. A 1:10,000 unadjusted germicide solution (pH 5.9) is bactericidal for *S. aureus* in less than 5 minutes of treatment. The activity of this solution is not destroyed by increasing the alkalinity to 10.9 but can be rendered inactive when the acidity approaches 3.7. An alkaline solution of pH 10.9 without alkyldimethylbenzylammonium chloride will not destroy the test organisms in 30 minutes. The effects of 1:10,000 of the quaternary ammonium germicide at the several pH ranges were duplicated when a 1:15,000 solution was used. A 1:25,000 germicide solution failed to destroy all the staphylococci in 15 minutes at pH 6.7 and 9.4. However, with the addition of alkali to pH 10 the solution became increasingly more active.

From these results it may be concluded that the bactericidal efficiency of alkyldimethylbenzylammonium chlorides is greater when in an alkaline range of pH. This data confirms the work of the earlier investigators on the same and many of the related quaternary ammonium salts.

TABLE 18

EFFECTS OF pH ON THE ANTISEPTIC EFFICIENCY OF A QUATERNARY AMMONIUM GERMICIDE* AGAINST *S. aureus*

QUATERNARY CONC.	ACID OR ALKALI PER 100 ML.	pH	BACTERIAL GROWTH AFTER MINUTES OF EXPOSURE 5	10	15
1:10,000†	0	5 9	−	−	−‡
1:10,000	0 10 ml. N/10 NaOH	9 6	−	−	−
1:10,000	0 25 ml. N/10 NaOH	10.2	−	−	−
1:10,000	0 50 ml. N/10 NaOH	10 4	−	−	−
1:10,000	1 00 ml. N/10 NaOH	10 9	−	−	−
Control (No Quaternary)	1 00 ml. N/10 NaOH	10 9	+	+	+§
1:10,000	0 10 ml. N/10 HCl	4 3	−	−	−
1:10,000	0 25 ml. N/10 HCl	3 7	−	−	−
1:10,000	0 50 ml. N/10 HCl	3 4	+	+	+
1:10,000	1 00 ml. N/10 HCl	3 2	+	+	+
Control (No Quaternary)	1 00 ml. N/10 HCl	3 2	+	+	+
1:15,000	0	6 6	−	−	−
1:15,000	0 10 ml. N/10 NaOH	9 4	−	−	−
1:15,000	0.25 ml. N/10 NaOH	10 0	−	−	−
1:15,000	0.50 ml. N/10 NaOH	10 3	−	−	−
1:15,000	1 00 ml. N/10 NaOH	10 6	−	−	−
Control (No Quaternary)	1 00 ml. N/10 NaOH	10 6	+	+	+
1:15,000	0 10 ml. N/10 HCl	4 3	−	−	−
1:15,000	0.25 ml. N/10 HCl	3 8	−	−	−
1:15,000	0 50 ml. N/10 HCl	3 5	+	+	+
1:15,000	1.00 ml. N/10 HCl	3 2	+	+	+
Control (No Quaternary)	1 00 ml. N/10 HCl	3 2	+	+	+
1:25,000	0	6.7	+	+	+
1:25,000	0.10 ml. N/10 NaOH	9.4	+	+	+
1:25,000	0 25 ml. N/10 NaOH	10.0	+	+	−
1:25,000	0 50 ml. N/10 NaOH	10 2	+	+	−
1:25,000	1.00 ml. N/10 NaOH	10.5	+	−	−
1:25,000	1.25 ml. N/10 NaOH	10.6	+	−	−
Control (No Quaternary)	1.25 ml. N/10 NaOH	10 6	+	+	+

* Alkyldimethylbenzylammonium chloride.

† Concentrations based on anhydrous germicide.

‡ Organisms destroyed, thus no growth in subculture medium.

§ Organisms survived, thus growth in subculture medium.

Sotier and Ward[221,222] were able to sterilize jute packing that is used in water mains which ordinarily, if untreated, would show the presence of coliform organisms for several months. Of particular interest to this section of the review is the fact that the quaternary ammonium germicide, *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride was most effective when used with a modified soda approximating in composition a Na_2CO_3-$NaHCO_3$ ratio of 1:1.39. The mixture used consisted of 5% of the quaternary ammonium germicide and 95% of the modified soda. The authors pointed out that the alkali portion of the formula is not to be considered as an inert carrier but that it served as a detergent and "activating agent" for the quaternary ammonium compound.

In studying the possible factors that may be responsible for the variations noted in the phenol coefficient tests on quaternary ammonium germicides, Stuart[77] found that the pH would materially affect the germicidal activity of these compounds. He noted that different quaternary ammonium compounds would hydrolyze in a different manner—some as salts of weak bases and strong acids, others as strong bases and weak acids. The pH may also vary from the time the original solution was prepared in water to several hours later. Stuart demonstrated that while a quaternary ammonium germicide will effectively destroy a test organism in 10 minutes by a dilution of 1:10,000 at pH 5.47, the same compound will give an effective kill in the same time period by a dilution of 1:18,000 when tested at pH 7.03.

GERMICIDAL ACTION IN ORGANIC MATTER

Hornung,[78] Domagk[20] and others[199,200] noted as early as 1935 that alkyldimethylbenzylammonium chloride was more active than most other germicides then available, even when tested in the presence of serum proteins. Hornung found that 0.01% of the quaternary ammonium solution, in the absence of serum, would destroy *S. aureus* in 15 minutes, whereas 0.03% was required in the presence of 16% human serum. In the same study, sublimate was effective in the absence and presence of serum in a concentration of 0.1% and Lysol in concentration of 0.7 to 0.8%. A concentration of approximately 1.5% of phenol destroyed the organisms in 10 minutes in the absence of serum, and 1.7% in its presence.

Schneider[87] observed that 10% blood serum would reduce the germicidal activity of alkyldimethylbenzylammonium chloride by 10%. A 1% serum medium, however, had no apparent antagonistic action on the germicide. Reference to the activity of the compound in the presence of serum was also made by Eschenbrenner.[223,224]

In this country, Dunn[22,172] was first to report on the germicidal action of alkyldimethylbenzylammonium chloride in varying concentrations of serum. These tests were carried out by substituting some of the water used in the dilutions for phenol coefficient tests with normal horse serum. Of a number of pathogenic Gram-positive and Gram-negative bacteria tested in the presence of 20% serum, *E. coli* was the most resistant organism, yet even here it was destroyed in 10 minutes by a 1:3,200 dilution at 37°C. At 20°C., a 1:200 dilution of the germicide in 50% serum proved lethal for *S. aureus* in 30 seconds. A 1:1,200 dilution killed the organism in the same concentration of serum in 10 minutes.

While studying the antibacterial actions of cetyltrimethylammonium bromide and related derivatives, Hoogerheide[147] found serum would cause a definite precipitate when added to the compounds. Furthermore, the addition of 2, 5, 10, and 20% serum to the test dilutions resulted in a decrease in phenol coefficient of 1200 at 37°C. to 380, 225, 150, and 62, respectively. Even with these marked reductions in values, he observed that a 1:2000 solution of cetyltrimethylammonium bromide was sufficiently active in the presence of 20% serum to kill a culture of *S. aureus* in 30 seconds. Probably the first reference to the germicidal action of a quaternary ammonium compound in the presence of milk may be found in the Hoogerheide publication. In 50% milk, the phenol coefficient of cetyltrimethylammonium bromide against *S. aureus* at 37°C. was reduced from 1200 to 56.

The effects of quaternary ammonium germicides on bacteria in milk were also studied by DuBois and Dibblee,[47] Johns and Pritchard,[225] and Mull and Fouts.[226] Their investigations were prompted by the fact that, inasmuch as the quaternaries were being used widely as sanitizing agents in dairy and milk processing plants, some unscrupulous dealers might intentionally allow a residual of the compounds to remain in the containers and thereby expect milk products with low bacterial counts. In the studies of DuBois and Dibblee[47] the addition to pasteurized milk of 9-octadecenyldimethylethylammonium bromide or alkyldimethyl-3,4-dichlorobenzylammonium chloride in a concentration of 1:5,000 prevented the formation of a curd when the samples were incubated at 37°C. for 24 hours. Alkyldimethylbenzylammonium chloride was markedly superior to the latter quaternaries, since it prevented the appearance of a curd even at a dilution of 1:25,000. The addition of 1:5,000 and

1:25,000 dilutions of alkyldimethylbenzylammonium chloride to pasteurized milk failed to prevent the growth of bacteria over a 48-hour period when incubated at 20° or 37°C. However, the acidity of the untreated milk was much higher than that of the milk containing 1:5,000 and slightly higher than that of the sample with 1:25,000 of the germicide. By increasing the concentration to 1:500 and 1:1,000 an immediate bacterial reduction of 90% and 60% respectively was obtained in raw milk. After incubation at 20° and 37°C., however, neither dilution showed any appreciable effect on the bacterial counts. The bacteria surviving and growing in the milk samples containing the higher concentrations of germicide were found to be primarily of the Gram-negative coliform types. Gram-positive cocci were inhibited by the same concentrations of alkyldimethylbenzylammonium chloride. By using the Hartley-Runnicles method for the chemical determination of quaternary ammonium compounds, DuBois found, moreover, that the procedure could be used to detect and estimate the germicide in the presence of milk.

In listing the attributes that are desired of a suitable chemical disinfectant McCulloch, Hauge, and Migaki[17] include the factor of maximum action of the compounds upon microorganisms in the presence of organic matter. These investigators confirmed the many observations of others that milk in concentrations as high as 50% will markedly reduce the germicidal activity of the quaternary ammonium disinfectants. Furthermore, it is not surprising to note their observations of the adverse effects of 20% cow manure and 50% bovine blood plasma on the antibacterial activity of these compounds.

Johns[120] reviewed the results of his studies on the comparison of the sanitizing effects of hypochlorites and quaternary ammonium germicides under conditions simulating those which may be encountered in a dairy plant. Both classes of compounds were found to retain their activity in the presence of "unexpectedly high concentrations" of skim milk when tested against *S. aureus.* In one instance alkyldimethylbenzylammonium chloride showed a 99% kill of the latter organism within 15 seconds in the presence of 10% skim milk.

Heineman[170] found serum would reduce the phenol coefficient values of alkyldimethylbenzylammonium chloride against several pathogenic bacteria. In the absence of serum and at 37°C., values of 271, 293, and 272 were obtained against *E. typhosa, S. aureus,* and *S. pyogenes,* respectively. The presence of 10% serum in the medication tube reduced the figures to 72, 154, and 129, in the order given.

In determining the critical killing dilution of cetylpyridinium chloride for a large variety of bacteria, Quisno and Foter[213] also noted that a definite increase in concentration of the germicide was required to destroy the organisms in the presence of 10% serum. For most Gram-negative rods the range of germicidal dilution was 1:2,000 to 1:19,000. *E. coli* and *Ps. aeruginosa* were the only bacteria in this group requiring a concentration greater than 1:1,000 to give 100% kill. For several varieties of cocci the range of killing dilutions was from 1:12,000 to 1:20,000. They noted also that the use of their germicide in the presence of human ascitic fluid, with a protein content below the level for normal beef serum, would give much higher critical killing dilutions than when the compound was tested in beef serum.

In preliminary studies leading to the use of cetyltrimethylammonium bromide in gloveless surgery and for the treatment of wounds and burns, Williams and his co-workers[214] found that 10% horse serum would reduce the activity of the germicide 9- to 27-fold. Nevertheless, appropriate concentrations of the quaternary ammonium compound were more active than soap in removing dirt, congealed blood, oil, grease, and tar from cuts and burns and still maintained asepsis in the cleaned areas.

In their search for preservatives for syphilitic serum, Croft and Smith[227] found alkyldimethylbenzylammonium chloride, penicillin, acriflavine, and a large series of other compounds to be ineffective as bacterial growth depressants for undiluted serum. Only an organic mercurial appeared to give the desired preserving action in a concentration which would not interfere with the serological activity of the serum. Additional references to the effects of proteins on quaternary ammonium germicides may be found in the publications by Hettche[88] and others.

Unlike the reports of the incompatibility of quaternary ammonium germicides and organic matter is the singular observation of Ordal and Rucker[228] who found that the germicidal action of some members of a homologous series of alkyldimethylbenzylammonium chlorides was enhanced by the presence of organic materials containing nitrogen. Since this note appeared in an abstract of their studies on the use of the compounds in controlling gill disease in salmonoid fishes, we await with interest a detailed account of their findings, particularly on the quaternary-protein phase of their investigations.

AGAR-PLATE TEST

There are occasional references in the literature to the use of the F.D.A. agar-plate or cup-plate tests (Circular 198), originally described by Himebach, for testing quaternary ammonium compounds. This test procedure serves as a useful means for testing the antiseptic properties of "salves, dusting powders, creams, plasters, pads, adhesive tape," etc. In other words, an index to the antiseptic properties of germicides in water-immiscible powders, creams, etc., may be determined by the agar-plate method. The test in some instances has also been used to determine the penetrative and inhibiting properties of certain liquid or water miscible germicides.

By merely incorporating alkyldimethylbenzylammonium chloride uniformly in an agar medium to give a final dilution of 1:10,000 of the germicide, Schneider noted that the growth of *S. aureus* was completely inhibited. *B. subtilis* (Hay bacillus) was inhibited in the same medium by a 1:80,000 dilution of the quaternary ammonium compound. Using the F.D.A. agar cup-plate procedure, Heineman[170] was able to demonstrate inhibition of *S. aureus* in plain agar and serum (10%) agar by a dilution of 1:100,000 alkyldimethylbenzylammonium chloride. *E. typhosa* was inhibited by a 1:5,000 concentration of the germicide but not by 1:10,000. *S. pyogenes* required a concentration of approximately 1:100 before inhibition could be observed. Inhibition was also obtained on 1:1,000 of the compound in cottonseed oil, mixtures of 50% mineral oil and 50% cottonseed oil, or a mixture of 5% lanolin and 95% mineral oil when tested against *S. aureus* in plain or serum-containing media. Heineman pointed out that one need not expect a correlation in the agar cup-plate and phenol coefficient tests.

Deskowitz[199] tested alkyldimethylbenzylammonium chloride by the agar cup-plate method by selecting the dilution that was 10 times the lowest concentration that inhibited the growth of *S. aureus* in broth (1:320,000). By using a 1:32,000 dilution of the germicide, a zone of 3.2 mm. was obtained against the latter organism in a 10% serum-agar medium. In the same test a 1:20,000 dilution of sodium 4-nitroanhydrohydroxymercuriorthocresol[1] gave a zone of 5 mm.; 1:2,000 hexylresorcinol gave 4.5 mm.; and phenol, a zone of 5.0 mm.

Hoogerheide[147] studied the homologous series of alkyltrimethylammonium bromides by the "penicillinder" procedure, which consists of adding the test solutions to small glass cylinders placed on the surface of an inoculated agar medium. The widest zone of inhibition against *S. aureus* was obtained with the C_{12} alkyl compound. The latter derivative was, however, less germicidal than the C_{14}, C_{16} and C_{18} analogues when tested in a broth medium. Hoogerheide refers, therefore, to the unreliability of the agar-plate method if it is used solely in the evaluation of the antibacterial activity of certain germicides.

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[1] See page 202 for trade name and distributor.

Tobie and Ayers[229] attribute the rate of diffusion in agar as the factor responsible for the discrepancies in the agar plate and phenol coefficient testing of quaternary ammonium and other germicides. They noted in cup-plate tests that a 1% solution of dodecyltrimethylammonium bromide gave a clear zone of 6 to 7 mm., whereas the octadecyl derivative gave but 2 mm. against *S. aureus.* Yet the phenol coefficient values on the two solutions were 78 and 160 respectively.

A unique method for determining the penetrating power of germicides has been described by Gutfeld.[230] The procedure consists of dipping sterile cotton strings in a culture of test organisms, allowing the culture to dry in the incubator and then pouring gelatin or agar over the contaminated string in a Petri dish. After the medium has solidified, the germicide solution is poured over the surface of the agar. At variable time intervals the "embedded germ carrier" is removed from the solid medium, rinsed in sterile water, and incubated at 37°C. in nutrient broth. By this test procedure Gutfeld demonstrated that it took more than 15 days for a 1% phenol solution to penetrate a layer of 3% gelatin, 2.4-mm. thick, to kill organism at 10°C. In a "direct contact" test, phenol detroyed the same organism between 4 and 23 hours at 28°C. *E. coli* was killed in the latter test within 25 minutes. Cotton strings were contaminated with *Tr. gypseum* and also embedded in 1% agar. A 1:1,000 as well as a 1:5,000 solution of alkyldimethylbenzylammonium chloride flooded over the surface of the agar plates showed no evidence of kill even after 8 days' exposure of the germicide on the embedded culture. By the direct method, however, the culture was destroyed by the 1:5,000 concentration within 35 minutes.

A different interpretation for the gross discrepancies noted in the results on tests of quaternary ammonium salts, when examined in agar media, as compared to liquid media, may be found in the studies of Quisno, Gibby, and Foter.[231,232,233] These investigators demonstrated that on testing quaternary ammonium germicides in the presence of 0.2% agar the lethal concentrations are three to six times greater than the lethal concentrations in the absence of agar. As examples, the germicidal dilution of alkyldimethylbenzylammonium chloride in plain broth was 1:38,000 against *S. aureus* at 37°C., and in the presence of 0.2% agar solution but 1:8,000. In the same test, *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride, cetyltrimethylammonium bromide and cetylpyridinium chloride were reduced from 1:32,000, 1:80,000, and 1:85,000 to 1:10,000, 1:15,000, and 1:14,000, respectively. Data was also presented to show that undissolved granular agar in the broth reduced the germicidal activity of the quaternary ammonium germicides to about the same degree as did the dissolved agar medium. Chemical analysis for quaternary germicide, made on several concentrations of one of the salts that was treated with granular agar and the latter filtered from the solution, revealed a marked reduction, due to physical absorption, of the quaternary ammonium compound. Solutions that originally contained 0.02, 0.01, 0.005, and 0.0033% of the compound were reduced by treatment with agar to 0.003, 0.002, and 0.0016%, respectively. Quisno and his group concluded, therefore, that the agar-plate methods are not suitable for determining the germicidal activities of quaternary ammonium solutions or products containing these compounds. Physical absorption rather than unequal diffusion rates[117,229,230] is presumed to cause the variations and reductions in the germicidal action of the quaternary ammonium germicides in media containing agar.

Brown[234] and Brown, Krabek, and Skiffington[235] also found that quaternary ammonium germicides give extremely irregular and, in some instances, no demonstrable action on certain bacteria in the agar cup-plate test. Erratic results with questionable effects were obtained on an alcohol-acetone (tincture) solution of alkyldimethylbenzylammonium chloride against *Proteus vulgaris, E. coli,* and *E. typhosa.* An alcohol-acetone solution of *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride also showed but a suggestion of a bactericidal action against 9 of 12 varieties of bacteria in the presence of an agar medium.

The lack in correlation between agar-plate results and serial dilution tests in broth is not solely inherent in studies on quaternary ammonium germicides. In studies on the relation of the effects of chlorine to bacterial death, Mudge and Smith[236] found close agreement between the end-titer of the serial dilution and the plate counts made before the addition of chlorine. After chlorine treatment, however, this correlation no longer was in evidence. While the agar plates showed the expected decrease up to 99%, the serial dilution showed growth in as great dilutions after the action of chlorine as before. The authors concluded their remarks with the following statement: "There are apparently few tests that can be relied upon where bacterial death is concerned."

INFECTION-PROTECTION TESTS

Probably the most accurate means for determining the critical killing dilutions or distinguishing between the bacteriostatic and the bactericidal effects of germicides and antiseptics may be found in the various "infection-protection" tests reported in the literature. While these tests, in animals or chick embryos, serve to give a true bacteriological differentiation between inhibition and complete kill of the pathogenic bacteria, the methods are not readily applicable to the routine testing of a particular germicide due to the expense and training necessary in carrying out the procedures.

Neufeld and Schutz[238, 239] treated suspension of *Pasteurella* and *S. typhimurium* organisms with alkyldimethylbenzylammonium chloride and found that concentrations of 1:8,000 and 1:4,000, respectively, would kill these bacteria within a period of 2 minutes. A 1% soap solution was used to neutralize the germicidal action of the quaternary ammonium compound prior to inoculation of the test suspension into the peritoneal cavity of mice. Survival of the animals to both experimental infections was considered evidence of a bactericidal action of the germicide upon the test organism.

Nungester and Kempf[237] described an infection-prevention method in which mouse-virulent Type I pneumococci or *S. pyogenes* are used as test organisms. The procedure involves the inoculation of the tail of a normal mouse with one of the bacterial cultures and then dipping the tail in a solution of disinfectant. One-half inch of the tail is cut off and inserted through an incision into the peritoneal cavity of the same mouse. The incision is properly closed and the animal observed for 7-10 days. Mice dying from the infection are autopsied and a sample of heart blood streaked on blood agar medium. Mortality rates in the pneumococcus tests were found to be 92% and 100% for a 1:1000 aqueous solution of *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride and for a 1:1000 aqueous solution of sodium ethylmercurithiosalicylate,[m] respectively. Using an alcoholic-acetone (tincture) solution of the two germicides the mortality rates were 72% and 62%, respectively. While the latter figures are lower than those noted when the aqueous solutions were used, the alcohol-acetone vehicle without germicide was found to be as effective as the tinctures. A 2% aqueous iodine solution and a 2% alcoholic iodine solution gave mortality rates of 5% and 0%, respectively. Against *S. pyogenes* the mortality rates of tinctures of *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride and sodium ethylmercurithiosalicylate along with the control alcohol-acetone solution were 90, 96, and 100% respectively. The aqueous and alcohol solutions of iodine in the same test were again found to be more effective with mortality rates of 48 and 36%.

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[m] **See page 198 for trade names and distributors.**

Using the Nungester and Kempf infection-prevention test, Pierce and Tilden[240] evaluated the germicidal activity of several common disinfectants against pneumococci. Of the compounds tested, phenol and iodine, each in 2% aqueous solution, and dodecyldimethylbenzylammonium chloride in 1% aqueous or tincture were found to be almost completely effective in killing the pneumococcus. By decreasing the concentration of the quaternary ammonium germicide to 0.1%, the aqueous solution protected only 58% and the tincture protected 78% of the animals. A 0.1% aqueous solution of sodium ethylmercurithiosalicylate gave virtually no protection to the animals. Tests were also carried out by Pierce and Tilden in which *M. tuberculosis* was used following their examination by the standard phenol coefficient whereupon they were injected intraperitoneally into guinea pigs. A 1% solution of phenol, 2% solution of saponated cresol, and 1% tincture of dodecyldimethylbenzylammonium chloride were found to give a complete kill of the acid-fast organisms as determined by this infection-protection procedure.

Instead of using the mouse-tail procedure described by Nungester and Kempf, Sarber[241] smeared a loopful of test culture on the clean, shaven, and scarified abdominal area of the animal. The area was then treated with the germicide and 3 minutes allowed for its action. A small section of the area was excised, the peritoneal cavity opened, and the piece of skin placed inside. After proper closure of the incision the animal was observed for a period of seven days. By using this infection-prevention technic, Sarber found that a tincture of iodine protected 24 of 25 animals and a 1:200 tincture of cetylpyridinium chloride protected 9 of 25 animals. The alcohol-acetone control gave less protection than either of the two tinctures. A 1:1,000 tincture of sodium ethylmercurithiosalicylate and a 1:1,000 aqueous solution of hexylresorcinol saved 12 of 25, and 9 of 25 animals, respectively. In tests on nonfunctioning or dead tissue, the following order of protection was noted: tincture of iodine, 21 of 24; tincture of cetylpyridinium chloride, 11 of 24; tincture of sodium ethylmercurithiosalicylate, 9 of 24; and aqueous hexylresorcinol, 8 of 24.

Kenner, Quisno, Foter, and Gibby[242] evaluated the germicidal action of cetylpyridinium chloride by the F.D.A. method and also by injecting the treated bacterial suspension intraperitoneally in mice. A virulent strain of *S. typhimurium* was used as the test organism. The mice were anesthetized 5 hours after inoculation and specimens of heart blood and peritoneal fluid were cultured in F.D.A. broth. In the *in vitro* tests a 1:50,000 dilution of the germicide would effectively destroy all the test organisms. The mouse inoculation test showed that the killing dilutions were between 1:30,000 to 1:60,000 of the quaternary ammonium compound. The average killing concentration was 1:45,000. Their studies revealed, moreover, that the bacteria treated with the germicide were not detoxified by body fluids and tissues.

Studies were made by Schwyzer[243] on a quaternary ammonium germicide, myristyl-γ-picolinium chloride, in which the compound was tested for its ability to sterilize experimentally infected wounds in dogs. While the test was by no means intended to be included as a routine measure for evaluating the germicide, certain salient points in the study may, nevertheless, be noted here. Wounds infected with 6,000,000 organisms per ml. could not be sterilized with the quaternary ammonium compound when the latter was used as an irrigant immediately after surgical manipulation of a foreign body (bone graft) implanted in the wound. However, if the inoculum was reduced to 4,000,000 bacteria per ml., the wound was sterilized by the germicide. Myristyl-γ-picolinium chloride was valueless if the treatment was delayed for 18 hours after introduction of the organisms.

Green,[244] and Green and Birkeland[245,246] used the developing chick embryo as a means of evaluating quaternary ammonium germicides and other wound disinfectants. In their first series of studies, the technic of Goodpasture and Buddingh[247] was used for preparing the windows in eggs and subsequent inoculations with microorganisms. Using *S. aureus* as the infecting organism, cetylpyridinium chloride was found capable of definitely reducing the degree of infection of the living chorioallantoic membrane of developing chick embryos. A solution of iodine and chloroazodin had little or no effect; phenol, sodium 4-nitroanhydrohydroxymercuriorthocresol and sodium ethylmercurithiosalicylate had no demonstrable effect on the infection. Of 17 cationic detergents studied, Green[244] found that 14 had definite therapeutic value in protecting the chick embryos from *S. aureus* infection. By using this infection-protection test, he observed variation in chain lengths of the alkyl radical did not result in any great differences in therapeutic activity when the compounds were tested in dilutions of 1:2,500 and 1:5,000. However, in dilutions of 1:10,000 the dodecyl derivative appeared to be slightly less effective than the hexadecyl (cetyl) and octadecyl (stearyl) compounds. The anionic surface-active agents studied gave no demonstrable effect against the staphylococcal infections even when the detergents were used in concentrations of 1:400 to 1:5,000.

A variety of wound disinfectants was evaluated in a subsequent study by Green and Birkeland[246] by the chick-embryo method. Sterile membranes were obtained with penicillin, cetylpyridinium chloride, α-carbomethoxypentadecylmethylpiperidinium bromide, alkyldimethylbenzylammonium chloride, and *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzyl chloride. The latter four compounds gave a definite curative effect against the *S. aureus* infections in a dilution of 1:10,000. While cetyltrimethylammonium bromide had only a slight curative effect in the 1:10,000 dilution, the 1:5,000 gave evidence of a definite protective action. In the same study, iodine was ineffective in dilutions of 1:5,000 and 1:10,000, whereas a slight reduction in the numbers of bacteria recovered from the membranes was noted when a 1:2,500 dilution was used. The results obtained by penicillin were striking in that dilutions of 1:500, 1:1,000 and 1:2,000, or greater, were as effective as the best germicide tested. Neither of the two mercurial antiseptics studied, sodium ethylmercurithiosalicylate and sodium 4-nitroanhydrohydroxymercuriorthocresol, nor phenol or dichloroazodicarbonamidine were effective in the tests in all the dilutions studied.

Spaulding and Bondi[248] have more recently included a toxicity test along with the infection-prevention test for evaluating germicidal agents. In both procedures, the mouse-tail test is used to determine the IP/50, or infection-prevention of 50% surviving animals, and T/50, the dilution of germicide alone that just permits survival of 50% of the animals. Type I pneumococcus was used as the infecting organism in the IP/50 determinations. In this study, several compounds were evaluated among which were tyrothricin and two quaternaries, 2-methyl-5-trimethylammoniumbenzyldodecylethermethosulfate and alkyldimethylbenzylammonium chloride. The IP/50 values for the compounds in the order given were 1:30,000, 1:200, and 1:200, respectively. The T/50 ratios on the same compounds were 1:2.5, 1:100, and 1:50, respectively. Therefore, the

IP-T indices were 12,000, 4, and 2. These data reveal the high germicidal value of tyrothricin when allowed to act against pneumococcus Type I organisms on the tails of mice. Evidence is presented by Spaulding and Bondi to show that tyrothricin is more active against pneumococci than against *S. aureus*. When tested *in vitro* in the presence of 10% serum, the antibiotic attains an end point of killing concentrations at 1:4,000,000 for the pneumococcus and but 1:2,000 for the staphylococcus. The two quaternary ammonium compounds on the other hand showed considerably less variation in specificity against these Gram-positive cocci, being effective in 1:64,000 for the pneumococcus and 1:4,000 to 1:8,000 for the staphylococcus.

Using a technic similar to the procedure of Green and Birkeland, Gershenfeld and Witlin[249] more recently determined the toxicity indices of a representative series of bactericides. They defined the toxicity index as the ratio of the actual amount of germicidal agent contained in each milliliter of the highest dilution required to kill *S. aureus* in 10 but not in 5 minutes at 37°C., to the actual amount of the agent required to kill a nine-day-old chick embryo within 24 hours. Examination of the data reveals that iodine solutions gave the most favorable toxicity indices of all the compounds tested. A value of 0.0125 was obtained on an aqueous iodine preparation and 0.18 on the tincture. The compound next in order of effectiveness was alkyldimethylbenzylammonium chloride with a value of 0.0377 on an aqueous solution and 0.925 on the tincture. In these evaluations, the smaller the toxicity index, the more nearly perfect is the germicide. The data of Gershenfeld and Witlin also reveals that indices as high as 16.5 may be obtained on tinctures of organic mercurials and 10.4 on hypochlorite solutions. They demonstrated, moreover, that the high apparent germicidal activity of mercurial antiseptics could be markedly or completely nullified by using thioglycollate medium in the bacteriological tests.

SANITIZING TESTS

The word *sanitize* is a new term that has been added to the nomenclature describing a means of disinfection. McCulloch[250] defines the word as follows: "Sanitize means to render sanitary. The term conveys the idea of disinfection without any residue harmful to subsequent users of the article or product, as well as the elimination of the contamination which might be esthetically objectionable. Thus, it can be said that dishes are *sanitized* by adequate cleaning and bactericidal treatment." Since the term "disinfectant" is described by McCulloch also as "A *disinfectant* destroys infectious agents and *disinfection* is the act of destroying infectious agents," it would appear that *sanitize* and *disinfect* are words that could be used synonomously in describing a condition resulting from a bactericidal treatment. It is not implied, therefore, that one must expect a 100% kill in all instances where a disinfecting or sanitizing agent is used. This is particularly true in the fields of general disinfection or in the application of chemical disinfectants to inanimate objects. To render an object absolutely free of any living or potentially living form of life by the use of chemical disinfectants, is virtually impossible. The various modifications proposed in the testing of germicides and antiseptics by numerous investigators previously mentioned in this article and elsewhere serve to indicate the confusion that exists at present in defining the term "100% kill" when exposing solutions, eating, and drinking utensils, etc. to treatment with a chemical disinfectant or sanitizing solution (Klarmann and Wright,[156,157,158] Reddish,[211] Bernstein, *et al.*,[212] Weber and Black,[251] Cade[186]).

Use of the word *sanitize* has gained popularity with public health officers and laboratory workers engaged in controlling sanitary conditions in eating and drinking establishments, in the dairy industry and milk products plant, bakeries, fisheries, and other food processing plants. Reference to the word *sanitization* was made by Krog and Marshall[252] in their studies on the use of a quaternary ammonium germicide in maintaining satisfactory bacterial counts in the dish-washing rinse waters of eating and drinking establishments. One-minute exposure of the cleansed eating or drinking utensils to a 1:5,000 solution of alkyldimethylbenzylammonium chloride was found to be sufficient to reduce the bacterial count to below 100 bacteria per swabbed area. The latter value of 100 bacteria or less has been the accepted standard in swab tests, indicating a satisfactory bacteriological condition resulting from sterilization of eating and drinking utensils.

Krog and Marshall[253] also used alkyldimethylbenzylammonium chloride to sanitize milk-handling equipment in a dairy pasteurizing plant. Although in no instance were they able to demonstrate a 100% kill of the bacteria following the use of the germicide, an average percentage kill of 90% was considered to be evidence that the compound has a definite application in the sanitization of milk handling equipment.

Walter and Hucker[254] compared chlorine and a quaternary ammonium germicide in the sanitizing of beverage glasses. In only one instance were they able to demonstrate a 100% kill of all the organisms on the rims of used beverage glasses which were rinsed in a chlorine solution. Nevertheless, the bacterial counts in many cases were low enough to be considered insignificant and safe from a public health standpoint. Evidence of 100% kill was obtained in a large number of samplings in which alkyldimethylbenzylammonium chloride was used as the rinse solution. However, use of this compound failed to give complete bacterial destruction in all the tests made in the various drinking establishments.

MacPherson[255] used alkyldimethylbenzylammonium chloride in concentrations of 1:4,000 to 1:8,000 as a sanitizing rinse for eating utensils. Total plate counts of swabbings from tavern drinking glasses were for the most part below 100 per utensil. Furthermore, complete absence of living *E. coli* was noted in the test media even when the wash water was heavily polluted with coliform organisms. Reference is also made by MacPherson on the treatment of ice-cream scoops with 1:5,000 of the quaternary ammonium germicide. Before use of the latter, counts of 64 to "millions" of bacteria were obtained, whereas, after treatment, the figures were "less than 10."

Mallmann, Kivela, and Turner[208,256] compared the sanitizing action of several quaternary ammonium compounds with that of chlorine in field tests carried out in a drinking establishment. While in the majority of instances the glasses were completely sterilized by 180 ppm. of available chlorine (in the form of hypochlorite) as well as by a 1:6,400 dilution (180 ppm.) of one of several quaternary ammonium germicide solutions, in no single series was there evidenced a 100% kill of the bacteria on the glassware. Yet in those tests in which the number of bacteria did not exceed 100 per swab, the glasses were considered adequately sanitized regardless of the chemical agent used.

In developing a method for testing the sanitizing efficiency of quaternary ammonium and hypochlorite compounds, Johns[209] also used a 99.9% reduction in the numbers of organisms as the "end-point" for evaluating disinfectants. In his tests the 99.9% destruction was used to represent, in one instance, 91 colonies developing; in another, 114 colonies; and in still another instance, 62 colonies growing in the agar plate following treatment with a sanitizing agent.

Appreciation of the significance of a 99% or greater reduction in the number of bacteria, rather than considering a germicide valueless unless a 100% kill was obtained following treatment with a quaternary or other disinfectant, may be found in the publications by Mueller, Bennett, and Fuller,[84] and others. These three workers evaluated the germicidal activity and stability of many surface-active materials that are applicable for dairy use. The majority of the agents investigated were classed as quaternary ammonium compounds or alkyl aryl sulfonates. Compounds were considered "very effective" germicides if a bacterial survival of 0 to 0.5% was obtained. Those compounds that allowed a survival greater than 0.5% and up to 3% were considered "moderately effective." A third group, comprising compounds which permitted a bacterial survival greater than 3%, was considered "ineffective" from a practical standpoint. Of the 42 surface-active agents tested, Mueller and his co-workers found that only the quaternary and phosphonium compounds gave sufficient germicidal activity to be considered good sterilizers for dairy use. The properties of high solubility, relatively noncorrosive action, odorlessness, tastelessness, and colorlessness also favored the use of the quaternary ammonium germicides over the other classes of disinfectants studied.

ACTION AGAINST MYCOBACTERIA

Early references to the effects of a quaternary ammonium germicide against the tubercle bacillus may be found in the publication by Domagk,[21] Hornung,[78] and later by Nagel.[215] Domagk included the tubercle bacillus in the list of organisms that he considered were unaffected by "practical" dilutions of alkyldimethylbenzylammonium chloride. Hornung also casually referred to the ineffectiveness of the quaternary ammonium compound against the tubercle bacillus. He inferred that the germicide's inactivity against the "waxy" organisms may be explained by the fact that anionic soaps will also reduce or neutralize completely the bactericidal action of the cationic compound.

Harris and Bunker[257] studied the effects of surface tension depressants upon the type of bacterial growth that will develop in a nutrient medium. They observed that when the bacilli were grown for 10 days in a medium with a surface tension of 44 dynes per cm., the organisms lost their virulence for guinea pigs. They postulated that the surface tension depressants caused the tubercle bacillus to become "wetted," thereby growing diffusely instead of in pellicle form in the medium. Thus, when the organisms are injected into the animal body, they are more readily penetrated by antibodies or bactericidal substances and destroyed. Although no mention was made of the action of cationic (quaternary ammonium) compounds in these studies, data were presented to show the effects of an anionic surface tension depressant upon the tubercle bacillus. Injection into guinea pigs of a mixture of equal amounts of 2% sodium ricinoleate and tuberculous sputum that had been allowed to stand for several hours failed to produce tuberculosis in the animals. Reference has already been made of the studies of Pierce and Tilden[240] who found that a 1% tincture of dodecyldimethylbenzylammonium chloride would give a complete kill of *M. tuberculosis* as determined by guinea-pig inoculation tests.

Freedlander[153] studied the *in vitro* effects of several anionic wetting agents and a cationic germicide, alkyldimethylbenzylammonium chloride, upon the growth of a nonvirulent strain of tubercle bacillus. The quaternary ammonium compound was found to be markedly superior to the anionic wetting agents in suppressing the growth of the organism. Complete inhibition of growth was obtained by a 1:80,000 dilution of alkyldimethylbenzylammonium chloride, while partial inhibition was noted even in a dilution of 1:400,000. The anionic compounds gave complete inhibition of the acid-fast organisms in dilutions ranging from 1:1,000 to 1:5,000 and partial inhibition in dilutions of 1:10,000 to 1:40,000.

Drea[258] determined the *in vitro* effects of various organic substances on the H-37 strain of human tubercle bacillus when tested in a synthetic medium. The most active quaternary ammonium compounds studied were alkyldimethylbenzylammonium chloride and cetylpyridinium chloride. These germicides inhibited growth of the acid-fast organism in a dilution of 1:10,000. In the same study, *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride exerted the same effect in a concentration of 1:1000. Results comparable to those obtained with the first two quaternaries mentioned were noted in tests using sodium 4-nitroanhydrohydroxymercuriorthocresol and sodium ethylmercurithiosalicylate.

Katz and Lipsitz[259] studied the effects of three types of surface-active agents upon the growth of *M. smegmatis.* The compounds included a quaternary ammonium salt prepared by reaction of dimethylsulfate with the stearyl ester of unsymmetricaldiethylethylenediamine, and two anionics, monosodium sulfonate of butylated-*o*-hydroxy-diphenyl and the *N*-methyl-tauride of oleic acid. The quaternary gave complete inhibition of growth of the test organism in a dilution of 1:2,000 while a concentration of 1:1,000 was required for complete destruction of the mycobacterium. The second compound showed inhibition in a dilution as high as 1:12,000 while a 1:2,000 dilution gave complete kill of the organisms. The last surface-active agent mentioned showed evidence of inhibition and destruction of the culture in a concentration of 1:2,000.

Quisno and Foter[213] found cetylpyridinium chloride would kill *M. phlei* in 10 minutes in a dilution of 1:1,500 in the absence of serum and in a 1:1,000 dilution in the presence of 10% bovine serum. Yegian, Budd, and Middlebrook[260] determined the effects of several germicidal agents upon sulfonamide-resistant and sulfonamide-sensitive strains of *M. ranae* by the agar cup-plate technic. A 1:1,000 solution of alkyldimethylbenzylammonium chloride gave a zone of inhibition of 47 mm. against the sulfa-resistant strain and 41 mm. against the sulfa-susceptible strain. Acriflavine produced zones of 50 and 44 mm. against the resistant and sensitive strains, respectively. A 1:833 alcohol-acetone (tincture) of sodium 4-nitroanhydrohydroxymercuriorthocresol gave zones of 68 and 53 mm. against the resistant and susceptible strain of the acid-fast organisms.

The high bacteriostatic activity of alkyldimethylbenzylammonium chloride against acid-fast mycobacteria has also been noted in tests carried out by the writer and his associates.[261] A 1:1,000,000 dilution of the germicide inhibited the growth of a human strain of tubercle bacillus in a fluid medium for a period of three weeks, while a concentration of 1:100 was required to kill a heavy suspension of the organism in 2 hours and 1:500 in 3 hours (F.D.A. procedure using Petragnani's slants for transplants). A strain of *M. avian* was inhibited by a 1:200,000 dilution of alkyldimethylbenzylammonium chloride. A 1:500,000 concentration was bacteriostatic for *M. smegmatis,* while a 1:200,000 dilution proved lethal for this organism. *M. phlei* was destroyed by a 1:500,000 dilution of the quaternary ammonium germicide and a 1:4,000,000 dilution inhibited its growth.

Sporicidal Properties

An early reference to the possible effects of a quaternary ammonium germicide (alkyldimethylbenzylammonium chloride) against spore-forming bacteria was made by Domagk.[21] In a footnote of his original publication the statement is made of the ineffectiveness of "practical" dilutions of the compound as well as bichloride of mercury, alcohol, cresol compounds, and other disinfectants upon spores of *B. anthracis* and the organisms producing "gas gangrene" (anaerobic clostridia). No laboratory data were given by Domagk to substantiate his statements. Hornung[78] presented laboratory evidence which showed a definite sporicidal action of alkyldimethylbenzylammonium chloride against *B. anthracis* spores. A 10% solution of the germicide destroyed the latter spores in less than 15 minutes while a 1:200 dilution required between 30 and 60 minutes for complete destruction of the highly resistant cells. In the same test, a 1% sublimate solution was effective between 9 and 24 hours, undiluted Lysol between 34 and 48 hours, and 5% phenol between 6 and 9 hours. Another reference to the effects of a quaternary ammonium germicide against *B. anthracis* spores may be found in an abstract by Green and Birkeland.[262] Although no actual figures were given, these authors indicated that cetylpyridinium chloride is an effective and practical germicide for bacterial spores of the organism mentioned, as well as for spores of *B. subtilis* and several anaerobic clostridia.

B. subtilis appears to have been the spore-forming organism of choice for determining the sporicidal action of quaternary ammonium germicides. Berge[263] found that concentrations of 1:200, 1:1000, and 1:2000 of alkyldimethylbenzylammonium chloride would destroy *B. subtilis* in ½, 1, and 3 minutes, respectively. In a broth-dilution test, Schneider[67] noted that a 1:200,000 dilution of the latter germicide would inhibit the growth of the organism (Hay bacillus) for a period of at least eight days, while Nagel,[215] and Luhr and Gutschmid,[264] found 1 to 2% of the compound was required to destroy the organism in 15 minutes and 24 hours, respectively.

Eschenbrenner[224] early demonstrated, however, the ineffectiveness of relatively high concentrations of alkyldimethylbenzylammonium chloride on spores of unidentified organisms in soil. Yet, in the same studies he found a concentration of 1:5,000 would kill spores of *B. anthracis* in less than 10 minutes. Sabalitschka[265,266] reported also on the inability of a 0.1% and 0.2% alkyldimethylbenzylammonium chloride to completely kill a suspension of spore-forming organisms, found in the soil, even after a 24-hour treatment.

Reference was made earlier in this report to studies of Sabalitschka and Maas[169] on the influence of temperature and pH on the sporicidal action of alkyldimethylbenzylammonium chloride. By using spores of unidentified bacteria from the soil, they were unable to demonstrate complete destruction of the cells by a 1:500 dilution of the germicide even when heated at 100°C. for 15 minutes. However, by increasing the heating period to 30 minutes in the presence of the germicide, complete destruction of the spores was obtained. In the absence of the germicide the suspensions survived heating at 100°C. for 3 hours. Thomann and Gschwind[167] observed that bacterial spores are not killed by a 1:100 (1%) solution of alkyldimethylbenzylammonium chloride at room temperature even after 2 hours but would be killed by simultaneous heating at 100°C. for 30 minutes.

Zeissler and Günther[267] were unable to confirm the data in a report of Brekenfeld and Kayser who claimed surgical instruments, heavily contaminated with heat-resistant bacterial spores, could be effectively sterilized by boiling for 15 minutes in 0.75% to 2% solution of alkyldimethylbenzylammonium chloride. In describing the procedure used by Brekenfeld and Kayser the former authors noted that a bacterial spore suspension was used which could resist boiling for a period of 12 hours. This suspension was mixed in garden soil to which was added some protein substance such as blood or egg albumen. This material served as the inoculum to contaminate the surgical instruments prior to treatment by boiling in the germicide solution.

In commenting on the work of Brekenfeld and Kayser, Zeissler and Günther point out the following objections to the test procedures: (1) The spore suspension used should resist boiling in plain water for 20 hours instead of merely 12 hours; (2) the spore suspension should constitute the numbers found in 1 gram of soil instead of an undefined or unknown amount of soil; (3) no neutralizing agent was used in the test to inactivate the residual or bacteriostatic amounts of quaternary ammonium germicide carried over in the nutrient medium.

By taking all the aforementioned factors in consideration, Zeissler and Günther were unable to demonstrate an absolute kill of all the spores in their test material even when the concentration of the quaternary ammonium germicide was increased to 10 and 20% and boiling was carried out for a period of 15 minutes. These investigators used liver-broth medium for "binding" or inactivating the quaternary ammonium compound in the subculture medium.

In a counter-report to the publication of Zeissler and Günther, Brekenfeld[268] stated that he and Kayser autoclaved 200 instruments, contaminated with spores, soil and protein, at a temperature of 120°C. for 20 minutes and found two of the instruments contaminated after this treatment. On the other hand, upon boiling 600 contaminated instruments for 15 minutes in 0.75% alkyldimethylbenzylammonium chloride, only one instrument showed the presence of residual contamination. In commenting on the use of 1 gram of soil in the test procedure, Brekenfeld inferred that his studies were designed to represent conditions which might be encountered under actual use of the germicide. Furthermore, since it is customary to brush or clean surgical instruments free of protein or "soil" after use, the amount of soil contamination used in the laboratory should have little bearing on the end results. The final point under consideration concerning the use of a neutralizing agent for the quaternary ammonium germicide was also not deemed necessary since figures were quoted by Brekenfeld to show that 100 instruments boiled in 0.03% of the quaternary gave 35% that were contaminated, and 100 instruments boiled in 0.01% of the germicide resulted in only 48% contaminations.

The controversial issues existing in our country today concerning whether or not the quaternary ammonium germicides actually kill bacteria or merely inhibit their growth were foreshadowed by the counter-reports between Zeissler and Günther, and Brekenfeld. In a final publication by the former[269] in concurrently running articles in the same journal, they stated that in their experience they knew of no bacterial spores that could withstand 8 minutes of heating at 120°C. Furthermore, the spore suspension used in their own investigations would withstand boiling for 50 hours yet were all destroyed by autoclaving at 120°C. for 20 minutes. Commenting on Brekenfeld's statement concerning laboratory tests versus field tests Zeissler and Günther remarked that the statement "sterilization" should mean 100% absolute kill under any condition to which a chemical or any other form of bacterial destruction was applied.

Dunn[22,172] reported that alkyldimethylbenzylammonium chloride in a broth dilution of 1:100,000 would prevent the growth of *B. subtilis* for at least four days. Using the F.D.A. technic for the *in vitro* testing of cetylpyridinium chloride, Blubaugh, Botts, and Gerwe[220] found that the compound compares in activity with other well-known germicides of mercurial, phenolic, and halogen types, against various bacteria including *B. subtilis* and several strains of fungi. Heineman[170] found a dilution of 1:500 (0.2%) of alkyldimethylbenzylammonium chloride would destroy a heavy spore suspension of *B. subtilis,* whereas a 5% phenol solution was ineffective even after 24 hours' exposure. The same organism was reported by White, Collins, and Newman[270] to be destroyed by a 1:500 aqueous solution of the compound in 5 minutes. While testing the bactericidal action of cetyltrimethylammonium and cetylpyridinium bromide, Williams and his co-workers[214] noted that the compounds in a broth dilution of 1:72,900 would destroy *B. subtilis* and *Cl. welchii* after

18 hours at 37°C. Concentrations of 1:218,700 of the germicides proved to be lethal for the spore-forming anaerobic organisms, *Cl. oedematiens* and *Cl. septicum.*

In studying the death rate of quaternary ammonium germicides on spore-forming bacteria, DuBois and Dibblee[271] found a 1:100 dilution of alkyldimethylbenzylammonium chloride would kill *Cl. tetani* spores in 20 minutes by 1:10 and *B. subtilis* in less than 30 minutes at 37°C. by a 1:10 dilution at pH 8.6. Of particular interest were their observations on alkyldimethyl-3,4-dichlorobenzylammonium chloride against *B. metiens* spores. Using the F.D.A. technic, they noted that a dilution of 1:10 (at pH 7.0) was required to kill all the spores. In the same test, dilutions of 1:5,000 to 1:20,000 of alkyldimethylbenzylammonium chloride resulted in an immediate kill of 60 to 75% of the spores and a 90% kill at the end of 6 hours. Furthermore, by using the F.D.A. procedure they were able to demonstrate that certain quaternary ammonium germicides would kill *S. aureus* in dilutions of the order of 1:50,000, whereas in death-rate studies over 90% of the organisms were destroyed immediately in dilutions as low as 1:300,000.

Deskowitz[199] found a 1:2,500 aqueous solution of alkyldimethylbenzylammonium chloride would destroy a culture of *B. subtilis* in 95 minutes at 37°C. The same compound was lethal for *Cl. welchii* in a dilution of 1:6,000 within 10 minutes. Lawrence[202] reported that a concentration of 1:500 to 1:600 of the same quaternary ammonium germicide will destroy a heavy suspension (0.5 ml.) of a 7- to 10-day culture of *B. subtilis* in less than 5 minutes when tested at 20°C. The latter author confirmed the data obtained by Deskowitz in studies on *Cl. welchii.* Lawrence,[202] Lawrence, *et al.*,[49] also demonstrated that dilutions of 1:50,000 to 1:200,000 of alkyldimethylbenzylammonium chloride and some of its derivatives would inhibit the growth, as well as destroy several strains, of pathogenic anaerobic clostridia in nutrient medium.

FUNGICIDAL TESTS

While the vegetative forms of fungi (molds) are relatively sensitive to chemical disinfectants, their spores are extremely resistant to practically all types of germicides, fungicides, and chemical agents in general. Although not specifically stating that spores of fungi were used exclusively in his studies, Dunn[22,172] presented data as early as 1936 on the fungicidal action of alkyldimethylbenzylammonium chloride. In the F.D.A. phenol coefficient tests at 20°C., values of 11, 30.8, and 400 were obtained with the quaternary ammonium compound against *Monilia albicans, Tricophyton interdigitale,* and *Microsporum lanosum,* respectively. The dilutions required to kill the fungi in 10 minutes, in the order given, were 1:10,000, 1:20,000, and 1:40,000.

In a subsequent report, Dunn[272] presented phenol coefficients and critical killing dilutions of a 50% alcohol—10% acetone tincture of 1:1,000 alkyldimethylbenzylammonium chloride upon a wide variety of fungi. Table 19 presents a reproduction of the data published by Dunn on these findings. Note is also made in the article to the destruction *E. inguinale, Ach. schoenleinii,* and *Act. gelanesis* by the 1:1,000 tincture of alkyldimethylbenzylammonium chloride.

TABLE 19

ACTION OF A 1/1000 TINCTURE* OF ALKYLDIMETHYLBENZYLAMMONIUM CHLORIDES AGAINST SOME FUNGI

(Dunn, *Proc. Soc. Exptl. Biol. & Med.*, 37, 661, 1938)

			AVER. HIGHEST DIL. DESTROYING TEST ORGANISM IN 10 BUT NOT 5 MIN.§			
	AVERAGE PHENOL COEFFICIENTS		PHENOL	AMMONIUM COMPOUND	PHENOL	AMMONIUM COMPOUND
TEST ORGANISMS	20° C.	37° C.	20° C.		37° C.	
Achorion schoenleinii	0.2	0.36†	1/87	1/17	1/140	1/50
Actinomyces gelanesis	1.0	1.3	1/130	1/130	1/205	1/275
Cryptococcus hominis‡	0.21	0.4		1/24		1/70
Epidermophyton inguinale	0.36	0.44	1/123	1/45	1/167	1/73
Microsporon audouini	0.074	0.12†	1/175	1/13	1/320	1/35
Microsporon lanosum	0.089†		1/90	1/8	.	.
Monilia albicans	0.17	0.60	1/77	1/13	1/93	1/55
Saccharomyces cerevisiae	0.09	0.15	1/100	1/9	1/135	1/21
Trichophyton interdigitale, No. 1	0.1	0.28†	1/60	1/6	1/90	1/25
Trichophyton interdigitale, No. 2	0.12	0.28†	1/63	1/7	1/90	1/25

* Alcohol-acetone—aqueous solution.

† One test only.

‡ Figures based on 1/1000 aqueous solution of the ammonium compound.

§ Dilutions of the 1/1000 solution.

In agar cup-plate studies Heineman[170] found a 1:1,000 aqueous solution of alkyldimethylbenzylammonium chloride would inhibit the growth of spores of *Tr. gypseum* to the extent of 20 mm. from the edge of the agar-cup in plain agar and 14 mm. in 10% serum agar. A solution of the germicide in various mixtures of oils gave a clear zone of 4 to 5 mm. against the same fungus. Heineman was also able to demonstrate that the addition of heavy spore suspensions of *Tr. gypseum* and *Mucor sp.* to a 1:500 aqueous solution of alkyldimethylbenzylammonium chloride would destroy the resistant cells in 5 minutes or less. In the same test a 1:120 phenol solution destroyed *Tr. gypseum* spores in 5 minutes, while the *Mucor* spores required a 28-hour treatment for complete destruction. In studies entirely independent from these, White and his co-workers[270] also found a 1:500 solution of alkyldimethylbenzylammonium chloride to be lethal for spores of *Tr. gypseum* and *Mucor sp.* within 5 minutes.

Joslyn, Yaw, and Rawlins[85] found a 1:1,000 aqueous solution and a 1:500 alcohol-acetone tincture of *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride would destroy the following molds in 5 minutes: *Actinomyces sp., Epid. rubrum, M. lanosum* var. *felineum, Mon. albicans, Tr. gypseum, Tr. interdigitale,* and *Tr. violaceum.* No mention was made of the critical killing dilutions that were required for complete destruction of the fungi by the two solutions.

Included among the various organisms that were tested with cetylpyridinium chloride by Quisno and Foter[213] were several strains of yeast and molds. When tested at 37°C. *Candida albicans, Cryptococcus neoformans, Tr. mentagrophytes* and *Microsporum canis* were destroyed by dilutions of 1:37,000, 1:61,000, 1:36,000, and 1:34,000, respectively. When tested in the presence of 10% serum, considerably higher concentrations of cetylpyridinium chloride were necessary for complete destruction of the fungi. In the order named, 1:3,500, 1:6,000, 1:3,000, and 1:5,000 of the quaternary ammonium germicide were found to be the critical killing concentrations against the organisms in the presence of serum.

Lawrence[202] determined the phenol coefficient values of alkyldimethylbenzylammonium chloride against spores of two plant-pathogenic and four-human pathogenic fungi. *Diplodia natalensis* and *Phomopsis citri,* the stem-end rot pathogens of citrus fruit seedlings, were destroyed by 1:20,000 and 1:40,000 of the quaternary ammonium compound. Of the fungi believed by some to be responsible for "athlete's-foot" infections in man, *Tr. rosaceum* was found to be the most resistant of the three trichophyta studied. A 1:6,000 concentration of the germicide was necessary to destroy the spores of the latter organism in 10 minutes at 20°C. Concentrations of 1:10,000 and 1:20,000 of the compound were effective against *Tr. gypseum* and *Tr. interdigitale,* respectively. *Microsporum audouini,* an etiological agent of "ringworm" infections in man, was found to be the most resistant organism of the series studied. A concentration of 1:2,000 was required to destroy the fungus in 10 minutes, as compared to a 1:80 dilution of phenol required to give a similar action.

Although not directly concerned with the lethal actions of germicides against fungi, Knight and Frazier[273] tested alkyldimethylbenzylammonium chloride and *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride and 35 other chemicals for their ability to prevent the growth of bacterial contaminants and still allow penicillin production by *Penicillium notatum* and *Pen. chrysogenum*. Only borax and boric acid could be used in concentrations high enough to prevent growth of contaminants in the medium without interfering with the development of the molds and production of penicillin. The two quaternary ammonium compounds mentioned, and the remaining diverse types of compounds, were too toxic for the molds in the ranges that prevented the growth of the contaminating organisms.

Hasseltine[274] recently compared the fungicidal action of a number of compounds against *Candida albicans*, an organism frequently found in the intestinal and vaginal cavities with or without symptoms, or evidence of mycosis. *In vitro* tests revealed that oxyquinoline sulfate, propyl-para-hydroxybenzoate, stearic acid, cetyl alcohol, triethanol amine, sulfamic acid, calcium sodium propionate, as well as the quaternaries cetyldimethylammonium chloride, cetyldiethylammonium chloride, and cetyl (di-*N*-butyl)-ammonium chloride held promise as fungicides for monilia which produce vaginal mycosis. On the basis of the favorable results obtained with the quaternary ammonium salts, Haseltine is at present conducting a clinical evaluation of 1% concentrations of the cetylammonium chloride compounds.

PROTOZOACIDAL TESTS

Fair, Chang, Taylor, and Wineman[275] studied the effects of a number of anionic and cationic synthetic detergents upon water-borne cysts of *Endamoeba histolyticum.* From their investigations, they concluded the cationic compounds were the most active of the two classes of detergents tested. Cetylpyridinium bromide and chloride, lauryldimethylbenzylammonium chloride, cetyldimethylbenzylammonium chloride, and alkyldimethylbenzylammonium chloride were cysticidal for spores of *E. histolyticum* in concentration ranges of 10 ppm. to 35 ppm. These results were obtained by allowing the germicide dilutions to act upon 30 cysts per ml. for 30 minutes at 23 to 25°C. Fair and his co-workers observed, furthermore, that the concentration needed became greater as the number of cysts were increased. However, the presence of organic matter and the hydrogen ion concentration had little influence on the activity of the cationic detergents upon cysticidal activity. Hexylresorcinol, the single nonionic compound studied, was cysticidal for the spores of *E. histolyticum* in 30 to 50 ppm., whereas the three anionic compounds studied failed to show evidence of destruction of the spores when used in concentrations as high as 1,000 ppm. Fair and his associates concluded their article by suggesting an exploration of the use of the cationic compounds for the sanitation of eating utensils; the safeguarding of shellfish; the cleaning and disinfection of contaminated vegetables and fruits that are consumed raw; the cleansing and disinfection of water mains, etc.

In an evaluation of the cysticidal effects of a large series (48) of heteropolar cationic (quaternary ammonium) antiseptics against *E. histolyticum,* Kessel and Moore[276] found that the majority of the compounds were effective in dilutions of at least 1:5,000; 20 of them effective in a dilution of at least 1:20,000; and three effective in dilutions over 1:80,000. In this study, as well as in the investigations of Fair,[275] survival of the treated cysts was based on the ability of centrifugated and distilled water-washed cyst suspensions to develop to amoebic trophozoites in a nutrient medium.

Johnson and Trussell[277] studied the *in vitro* effects of a large variety and number of compounds against *Trichomonas vaginalis.* Two quaternary ammonium germicides were included in the test series, *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride and alkyldimethylbenzylammonium chloride. The former compound killed the trichomonads in less than 10 minutes by a concentration of 1:1,600 while the latter proved to be ineffective in a 15-minute period in a 1:1,400 dilution.

Morgan and Campbell[278] examined 350 compounds for their trichomonacidal properties *in vitro* against the protozoa *Tr. foetus.* They considered the effective concentration to be that which killed the organism in 1-minute treatment. Among the commonly known quaternary ammonium germicides that killed the trichomonads in concentrations of 0.01% to 0.02% were alkyldimethylbenzylammonium chloride, cetylpyridinium bromide, and *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride. A concentration as low as 0.003% of cetylpyridinium chloride was reported effective against the protozoa in the study.

Quisno and Foter[213] briefly reported on the effects of cetylpyridinium chloride against a strain of *Tr. vaginalis in vitro.* They found that a 1:3,000 dilution of the quaternary ammonium compound would destroy the organism in the presence of 25% human serum within 10 minutes at 37° C.

Studies by Lawrence[202] also revealed the high protozoacidal activity of a quaternary ammonium compound against *Tr. foetus.* In this investigation note was made of the effects of various concentrations of alkyldimethylbenzylammonium chloride upon the general morphology of the trichomonads and upon the ciliary action of the undulating membrane of the organisms. Complete loss of motility, absence of ciliary motion, gross shrinkage of the cells, and complete fragmentation of the protozoa were all taken as toxic effects of the test substances upon the organism. Table 20 presents a reproduction of the results of this study. From the data given, it will be noted that a concentration of 1:1,000 alkyldimethylbenzylammonium chloride causes complete fragmentation of the protozoal cells. This destruction may be attributed to the surface tension depressing action of the germicide. A similar effect on the organism has been noted when anionic surface-active agents have been used in concentrations of 1:200 or greater. In all the media in which cell

TABLE 20

EFFECTS OF ALKYLDIMETHYLBENZYLAMMONIUM CHLORIDE AND PHENOL UPON TRICHOMONAS FOETUS

COMPOUND	FINAL CONCENTRATION	IMMEDIATE	EXAMINATION FOLLOWING	
Quaternary	1:5,000	Cells shrunken and nonmotile	.	. . . . .
	1:10,000	Cells round, few motile	30 min. Much fragmentation, Very few motile	1 hr. Mostly frag., Nonmotile
	1:20,000	50% normal 50% round Many motile	40 min. 75% frag., none motile	
	1:50,000	All normal and actively motile Normal shape	2 hr. All normal and actively motile	18 hr. Few normal and motile
Phenol	1:200	All nonmotile	. . . .	.
	1:1,000	All actively motile and normal	30 min. All actively motile and normal	. . .
Saline Control		All actively motile and normal	18 hr. All actively motile and normal	

fragmentation occurred, the surface tensions of the detergents were between 30 to 40 dynes per cm.

Upon decreasing the concentration of alkyldimethylbenzylammonium chloride to 1:5,000 in the test suspension, the trichomonads were markedly distorted. Unlike the oval, normal cell, the organisms seemed devoid of cytoplasm, as evidenced by the tightly twisted, cordlike appearance of individual protozoa. Many of the latter specimens were subsequently noted to have become fragmented when examined 30 to 60 minutes later. In the same study, high concentrations of phenol (1:200) completely immobilized the trichomonads (with no evidence of ciliary action and presumably dead), yet the organisms retained their normal morphological appearance. This same normal appearance was noted also when cells of *Tr. foetus* were exposed to protozoacidal concentrations of mercurial antiseptics.

An autolysis of protozoal cells, probably not unlike the action of quaternary ammonium germicides upon *Tr. foetus*, has been observed by Anderson[279] who exposed cultures of trypanosomes to the antibiotic, subtilin, which was found to possess surface tension depressant activity. The latter agent was also reported by Anderson to rupture the membrane of cultures of trophozoites of *Endamoeba histolyticum*. A photographic plate showing the actual rupturing of the cell membrane of the organism is presented in the latter publication. Brief mention may be made of the observations of Hauser, Phillips, and Vavruch[280] who noted that penicillin solutions also resemble soapy water and possess surface-active properties. These investigators found that the antibiotic carries an electric charge similar to soaps and, therefore, it has a tendency to cling to surfaces with an opposite charge. Using the ultraviolet light microscope technic, they were able to demonstrate that penicillin coats bacterial cells as evidenced by the bacteria that take on the fluorescent properties of the antibiotic when they are exposed to solutions of the latter agent.

One of the most extensive studies on the effects of quaternaries against protozoal cells and higher forms of invertebrates may be found in the papers by Taft and Strandtmann.[154,281,282] In one of their publications the sequence of events of the effects of alkyldimethylbenzylammonium chloride upon paramecia is vividly described. A 1:1,000,000 dilution of the germicide caused the movement of the organism to slow down, the extruding contractile vacuole extruded more than normally, and the cell finally ruptured allowing the protoplasm to flow into the surrounding fluid. Microscopic observations by Taft and his colleagues on the effects of alkyldimethylbenzylammonium chloride on higher invertebrates are presented in the following section of this review.

INVERTEBRATE TESTS

Search of the literature reveals but few references to the action of quaternary ammonium germicides on invertebrates. For this reason, the remarkable observations of Taft[154,281] and Taft and Strandtmann[282] will be reviewed in some detail. In the latter report the authors described the effects of alkyldimethylbenzylammonium chloride upon the larvae of mosquitoes. They observed that a concentration of 1:100,000 of the germicide would prevent the hatching of eggs of *Culex quinquefasciatus* and, furthermore, would kill the larvae of any stage and pupae. A concentration of 1:250,000 of the quaternary would kill first and second instar larvae, some third and fourth instars, and some pupae. Dilutions as high as 1:1,000,000 would effectively reduce the number of larvae.

Taft and Strandtmann found, however, that the *Aedes aegypti* mosquito was somewhat more resistant than *C. quinquefasciatus* to the action of alkyldimethylbenzylammonium chloride. While a 1:100,000 dilution killed first, second, and third instar larvae, the same concentration had little effect on pupae. A 1:250,000 solution killed approximately 80% first instar larvae, the remainder did not mature even for several weeks. While all these findings were based on laboratory studies, the suggestion was made that the quaternary ammonium germicide may be of some value in the field as a mosquito control agent.

In a subsequent publication, Taft[281] described the effects of alkyldimethylbenzylammonium chloride on the isolated heart of the horseshoe crab, *Limulus polyphemus*. He observed that concentrations of 1:1000 to 1:150,000 of the quaternary would stop the heart in systole within 1 to 40 minutes, depending upon the strength used. The action was found to be irreversible. It was impossible to determine whether the action of the germicide on the heart was purely muscular or nervous in origin.

A comprehensive review of the effects of alkyldimethylbenzylammonium chloride on various phyla invertebrates appeared in a third publication by Taft.[154] While the organisms reacted differently to the quaternary, this was not considered to be a phylogenetic difference but one due to the type of protective covering of the animal. He attributed the action of the germicide to be due, to a large extent, to its ability to lower surface tension and to its wetting properties. Microscopic examination of a culture of paramecia treated with alkyldimethylbenzylammonium chloride revealed the following order of sequence in reaction: The movement of the organism was slowed down, the trichocysts "shot out," and the extruding contractile vacuole extruded more than normally. The cell finally burst and the inner protoplasm flowed out of the animal, whereupon the latter finally disintegrated. This chain of events occurred in 1 minute with a 1:100,000 dilution of the germicide and in about 15 minutes with a 1:1,000,000 dilution. *Colpidium* and *Hypotriches,* other forms similar to paramecia, respond in a manner analogous to the cycle given.

With *Euglena* species, Taft observed a somewhat different response to alkyldimethylbenzylammonium chloride. The organism is more resistant to the germicide than the ciliates. Complete disintegration of *Euglena* does not occur even with a 1:20 dilution although immediate loss in motility can be noted and death occurs in 1 minute. In a 1:20,000 dilution of the quaternary, the cells lose their motility and assume a spherical shape in 20 minutes. Spherical forms may also be found when the organisms are exposed to 1:50,000 and 1:100,000 concentrations of alkyldimethylbenzylammonium chloride.

In studies with cultures of *Rotifers* and *Amphipoda,* Taft noted that these two species of animals were killed within a few hours by dilutions of the germicide as high as 1:100,000 to 1:400,000, respectively. Certain species of *Cyprids* were found to be quite resistant to alkyldimethylbenzylammonium chloride. *Planaria,* on the other hand, were changed from typical cells to "blobs" of protoplasm within 4 hours by a concentration of 1:100,000 of the germicide. A 1:1,000 concentration of the quaternary destroyed dog *Ascaris* in 30 minutes and the worms failed to respond to stimuli after 90-minute exposure to a 1:10,000 concentration of the germicide.

In extending the studies to include higher forms of invertebrates, Taft found that a 1:10,000 concentration of alkyldimethylbenzylammonium chloride would kill *Enchytrae albidus* worms in 15 minutes while a 1:1,000,000 would give a similar effect in 4 hours. Small starfish were killed by a 1:8,000 dilution of the quaternary in 1 hour and 15 minutes, and by a 1:400,000 solution within 24 hours. *Gastropod crepidula* were destroyed by a 1:2,500 solution of the germicide within 15 minutes and by a 1:8,000 dilution in 1 hour.

Reference is also made in the Taft article[154] to unpublished data on the effects of alkyldimethylbenzylammonium chloride on the barnacle, *Balanus*. Immersion of the cyprid larvae in dilutions of 1:10,000 to 1:80,000 of the germicide had little interference in the metamorphosis of the animals. Normal development of the adult organisms was seriously interfered with, however, when they were exposed to solutions of 1:10,000 to 1:25,000 of the quaternary ammonium compound. Bright refractive vacuoles were noted in the barnacles exposed to solutions up to 1:200,000. Upon first examination, very few of the animals showed feeding movements and all were dead within a short time after exposure to the germicide.

It is apparent that the studies of Taft and his co-workers represent a comprehensive and informative contribution to the literature on the effects and mode of action of quaternary ammonium germicides. Unfortunately, the lower forms of life (bacteria, viruses, etc.) do not lend themselves as readily to the detailed studies afforded by the higher forms of invertebrates used by Taft in his investigations.

VIRUCIDAL TESTS

An early reference to the possible effects of a quaternary ammonium germicide against viruses may be found in the publication by Kaiser[283] (1937). This article presented an economical means for preparing a vaccine against smallpox by the use of alkyldimethylbenzylammonium chloride to destroy contaminating bacteria and yet permit survival of the virus. No harmful effects of the germicide against the virus could be demonstrated. The disinfected lymph retained its high degree of activity following treatment, which Kaiser considered evidence of compatibility between the virus and the quaternary ammonium compound. He suggested a selective action of the latter against the Gram-positive contaminating bacteria in biologically prepared vaccines. The present-day routine use of alkyldimethylbenzylammonium chloride by Ducor[284] in our country, for the preparation of the scarified abdomen of calves prior to harvesting variola virus lymph, has also been attended with a minimum incidence of contaminating bacteria.

Maier[204] used alkyldimethylbenzylammonium chloride as a preservative for bacteriophage vaccines and venom solutions. He noted a 1:80,000 to 1:100,000 dilution of the germicide would kill *S. aureus* in less than 24 hours and 1:30,000 to 1:35,000 killed in less than 10 minutes at 30°C. A range of 1:100,000 to 1:800,000 inhibited the growth of the Gram-positive coccus. In contrast to the sensitiveness of the latter organism to the quaternary ammonium germicide, Maier found a 1:50,000 of the compound would not interfere with the reproduction of staphylococcal bacteriophage. Furthermore, the phage-disinfectant mixture was capable of reproduction of new phage even after three-month storage in a refrigerator. The 1:50,000 concentration was used, therefore, as a preservative for the vaccine, as well as for venom solutions.

Concentrations of 1:10,000 to 1:20,000 alkyldimethylbenzylammonium chloride have been used successfully by United States Navy Medical Units for the destruction of bacterial contaminants in throat washings and suspensions containing influenza virus. They originally found[285] that, while a 1:10,000 dilution of the germicide would destroy pathogenic bacteria, the same concentration would not inactivate Type A or Type B influenza virus when acting for 1 hour at room temperature. Incident to these findings were their observations that a 1:1,000 dilution of the germicide, in physiological salt solution, did not produce pulmonary irritation when given to mice by the intranasal route. Furthermore, 0.1-ml. aliquot of 1:5,000 to 1:100,000 alkyldimethylbenzylammonium chloride injected into the allantoic fluid of fertile chicken eggs did not kill the embryos. In subsequent studies[286] in which four methods were evaluated for the primary isolation of influenza virus from throat washings, adsorption on red blood cells and treatment with a 1:20,000 final concentration of alkyldimethylbenzylammonium chloride yielded the highest number of positive results.

Hammon and Reeves[287] demonstrated that a 1:10,000 dilution of alkyldimethylbenzylammonium chloride or sodium ethylmercurithiosalicylate, and 1:500 sodium sulfathiazole in normal rabbit serum, had no virucidal effect upon Western equine and St. Louis encephalomyelitis viruses. In the same study, mercuric cyanide 1:10,000 and phenylmercuric borate 1:50,000 affected neither of these viruses nor the Lansing strain of poliomyelitis virus. Phenylmercuric borate was used in place of the quaternary ammonium germicide for controlling bacterial contamination of the specimens since it was felt the latter germicide might interfere with certain *in vitro* tests due to its effects on surface tension.

Kalter, Mordaunt, and Chapman[288] described a method for the isolation of *E. coli* bacteriophage from sewage by means of cationic detergents. The detergents *N*-(alkyl acid ester of colaminoformylmethyl) pyridinium chloride, alkyldimethylbenzylammonium chloride, and cetylpyridinium chloride in a final dilution of 1:5,000 with or without primary incubation were recommended for the isolation of the bacteriophage from sewage and its concomitant bacterial flora.

Klein and Stevens[289] determined the *in vitro* and *in vivo* activity of 20 compounds, including cationic, anionic, and an unionized detergent as well as two commercial compounds against influenza A virus. The *in vitro* tests involved mixing the germicide dilutions with a virus suspension and, after standing at room temperature, a 0.05 ml. sample was inoculated intranasally into white mice. Deaths of the animals and degree of lobar consolidation were used as the index for evaluating the germicides. In the *in vivo* tests, the germicide dilutions were instilled intranasally in mice, or administered in the form of a spray in a closed chamber, just prior to and again following inoculation with influenza virus. The *in vitro* tests revealed the following: (a) of the quaternary ammonium compounds (cationics), a definite virucidal action was obtained with cetylpyridinium chloride, *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride and alkyldimethylbenzylammonium chloride; (b) in the series of anionic detergents, complete virucidal effect was obtained with a sodium salt of aryl-alkyl-ether sulfate,[n] sodium sulfate of 3,9-diethyl-tridecanol-6, sodium lauryl sulfate, and sodium myristyl sulfate; (c) hexylresorcinol was also found to be effective in these tests. The compounds listed were virucidal for the influenza A virus in dilutions ranging from 1:2,000 to 1:8,000 in 10 minutes, while dilutions of 1:500 to 1:2,000 gave a complete kill in 60 seconds. Although a high degree of virucidal activity was obtained in the *in vitro* tests, no protective action was afforded mice by the compounds when administered prior to influenza A virus inoculation. Klein and Stevens also called attention to the *N*-(alkyl acid esters of colaminoformylmethyl) pyridinium chloride where the alkyl represents carbon chain lengths of C_8 to C_{16}. Although the C_{12} derivative kills *S. aureus, in vitro,* in a dilution of 1:14,000 at 20°C., it had little activity against influenza virus in a concentration as high as 1:250.

In a continuation of the studies on the effects of synthetic surface tension depressants against viruses, Klein, Kalter, and Mudd[290] included bacteriophage, vaccinia virus, and the influenza A virus in their investigations. Confirmation was obtained on the high virucidal activity (1:4,000) of the several compounds given in their earlier paper. The same groups of compounds were also found to be virucidal for vaccinia virus. Furthermore, although the *N*-(alkyl acid esters of colaminoformylmethyl) pyridinium chlorides showed but a suggestion of an action against influenza virus, they proved, nevertheless, to be highly effective (1:4000) against vaccinia virus, *in vitro*. In the tests using bacteriophage, all the quaternary ammonium germicides tested proved to be lethal for *S. dysenteriae* and *S. albus* phage. Only one anionic compound showed evidence of an action against the phages mentioned. Of particular interest were the findings of Klein and his co-workers that, while the compounds mentioned showed a definite inactivating action against the latter bacteriophage, none of the agents tested gave any suggestion of a similar neutralizing effect against *E. coli* bacteriophage.

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[n] **See page 198 for trade names and distributors.**

While studying the effects of cationic detergents (quaternary ammonium compounds) in cotton rats, Toomey and Takacs[291] found that a 1:800 solution of cetyltrimethylammonium bromide would not neutralize nor destroy poliomyelitis virus. No data were given as to the possible action of the several other quaternary ammonium germicides on this same virus.

Krueger[292] compared the virucidal effects of a quaternary ammonium cationic detergent, alkyldimethylbenzylammonium chloride; two anionic detergents, sodium sulfate of 3,9-diethyl-tridecanol-6 and Ivory soap; and a compound containing nascent chlorine against Types A and B influenza virus. The detergents were mixed with a mouse-lung suspension of the virus, following which the material was instilled intranasally into mice. The quaternary ammonium germicide, in a concentration of 1:10,000, failed to show any evidence of virucidal activity. Since concentrations greater than 1:10,000 caused a precipitate of the virus suspension, Krueger considered it inadvisable to extend his studies to include the range of dilutions normally considered bactericidal for the quaternary ammonium compound (1:1,000 to 1:5,000). Concentrations ranging from 1:2,000 to 1:20,000 of the anionic detergent, sodium salt of 3,9-diethyl-tridecanol-6, did not completely inactivate a 5% suspension of influenza virus, although a 1:2,000 of the anionic agent reduced the activity of a more dilute suspension of the infecting material. The chlorine compound failed to inactivate a 5% suspension of influenza virus even when the former was used in a concentration of 1%. A 1% solution of Ivory soap did not completely inactivate a 5% suspension of virus although partial inactivation was noted after 40 minutes. Krueger, moreover, noted that the control animals receiving no virus, but the detergents alone, gave no evidence of deaths or pulmonary lesions following intranasal instillation of 1:1,000 of the quaternary ammonium germicide or of the anionic detergents, nor of 1:100 dilutions of the chlorine compound.

While Krueger was unable to demonstrate a virucidal action of the detergents in his studies, Stock and Thomas[293] found 0.001M solutions of alkyldimethylbenzylammonium chloride, dioctyl-ester of sodium sulfosuccinic acid, an alkyl sulfate, and sodium oleate to completely inactivate the PR-8 strain of influenza virus A. The first compound listed is a cationic quaternary ammonium compound while the remainder are anionic detergents. In the same study, several other anionic detergents failed to show evidence of a virucidal action against the virus. These wetting agents included the dihexyl, diamyl, and dibutyl esters of sodium sulfosuccinic acid.

Eaton, Corey, Van Herick, and Meiklejohn,[294] and Rose, Molloy, and O'Neill[295] on the other hand, found that alkyldimethylbenzylammonium chloride interfered with the development of influenza virus in amounts that controlled bacterial growth. Eaton and his group were unable to completely destroy all bacterial contaminants in throat washings of individuals by a 1:20,000 dilution of the quaternary ammonium germicide. Although higher concentrations were not specifically mentioned, they inferred that using concentrations greater than the amount given would result in an inactivation of influenza virus.

Knight and Stanley[296] determined the effects of 20 different chemicals upon the activity of a purified PR-8 strain of the influenza virus. The activity of the virus following treatment with the various compounds was determined by inoculation of the suspension in chick embryos as well as by intranasal inoculation in mice. Among the most active compounds studied were two quaternary ammonium germicides, *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride and alkyldimethylbenzylammonium chloride. Immediate inactivation of the virus was obtained by 0.5N and 0.05N concentrations of the latter compounds. Furthermore, complete inactivation was noted upon treating the influenza virus suspensions at 4°C. for a period of 2 weeks with concentrations as low as 0.0005N and 0.005N of the quaternary ammonium salts. The single anionic detergent tested, sodium dodecyl sulfate, completely inactivated the virus only after standing for a week or more. In the same study, Knight and Stanley found phenol would inactivate the virus promptly by a 0.5N concentration; however, the 0.05N solution required three weeks or more. A 0.01 and 0.05N (0.1% and 0.5%) phenol solution caused very little loss in activity in one month and one week respectively.

Tilley and Anderson[298] studied the effects of various disinfectants on the virus of Newcastle disease which was isolated from the lung tissue obtained from a natural outbreak of the infection in chickens. The investigation was carried out on 11-day embryonating eggs which were highly susceptible to infection by the virus. Their investigations revealed that 2% sodium hydroxide, 1% sodium orthophenylphenate, 1% liquor cresol saponatus U.S.P., and 0.1% of two quaternary ammonium compounds would destroy the virus of Newcastle disease within a period of 5 minutes. Calcium hypochlorite solution of low alkalinity was effective in a solution of 400 ppm., whereas in the same dilution sodium hypochlorite of high alkalinity failed to kill the virus. Formalin in a concentration as high as 4% failed to inactivate the virus after 30 minutes but was effective when allowed to act on the organism for 60 minutes. Fifty per cent isopropyl alcohol, 100% ethylene glycol and 4% sodium carbonate were found to be ineffective against Newcastle disease virus.

Based on the early observation of Kuhn and Bielig[297] that quaternary ammonium compounds would precipitate many proteins in an aqueous solution, Pfankuch and Kausche[299] studied the correlation of inactivation of tobacco mosaic virus and the virus attacking the plants of *Datura stramonium* with the degree of turbidity of these protein substances resulting from treatment with the homologous series of quaternary ammonium compounds. Data was presented to show that a correlation exists between the chain lengths of the paraffin alkyl group of the latter salts and the resulting flocculation, precipitation, and inactivation of the plant viruses mentioned.

MODE OF ACTION

Probably no other disinfectant or group of disinfectants has been studied more extensively with respect to its action against microorganisms than the quaternary ammonium germicides. In the introductory section of Jacobs'[12] early paper (1916), certain conclusions were drawn in respect to the correlation between chemical constitution of the hexamethylenetetramines and their action upon microorganisms. It was noted that the bactericidal nature of the compounds was principally attributed to the hexamethylenetetramine nucleus, whereas the degree of action was determined by the nature of the molecular groups added to the latter. The added groups were also found to be responsible for the partial specificity of certain of the preparations for particular bacterial species. This partial specificity did not appear to favor one species alone but all the species tested were found to be separately and specifically susceptible to some particular type of hexamethylenetetramine derivative. Jacobs demonstrated, moreover, that the added groups on hexamethylenetetramine were also the portions of the molecule responsible for serum incompatibility and toxicity of certain groups of compounds.

Although no evidence was presented to substantiate his report, Hornung[78] postulated that the inactivation of the antibacterial action of alkyldimethylbenzylammonium chloride by soap may be correlated with the failure of the germicide to act upon the tubercle bacillus. While not directly concerned with quaternary ammonium compounds, Cowles[152] was able to derive certain conclusions regarding the mechanism of action of a homologous series of alcohols. He observed that in the series of primary, secondary, and tertiary alcohols the germicidal power toward *E. typhosa* and *S. aureus* increased with increasing length of the carbon chain, and the surface activity likewise increased. The close relationship between germicidal activity and surface action, as revealed graphically, implied a biological relationship between these properties. His data suggested to him, moreover, that organic germicides may depend upon adsorption primarily and upon toxicity to bacterial protoplasm secondarily. Reference has already been made to the several publications on the correlation of the carbon chain lengths of the alkyl portion of quaternary ammonium germicides with the degree of antibacterial activities.

Harris and Bunker[257] included a quaternary ammonium compound, tetramethylammonium chloride, among the various agents studied for detoxifying action against bacteria. They defined "bacterial detoxification" as a process of neutralization of the toxic products of bacterial cells whereby the toxin is still able to stimulate the production of antibodies but no longer has a toxic effect in the animal body. Furthermore, detoxifiers neutralize the attack of bacteria without necessarily killing them, although inhibiting certain of their physiological processes and allowing the production of antibodies to continue. The quaternary ammonium compound that they studied had this action against tetanus toxin. The mechanism of action was believed to be due, not to the surface tension depressant action of the detoxifiers nor solely to any particular anion or cation, but to the configuration of the molecule as a whole.

Miller, Baker, and Harrison[79,218,219,300,301] determined the effects of quaternary ammonium germicides and other synthetic detergents upon the metabolism of bacteria. In these studies, washed suspensions of organisms were placed in appropriate buffers containing 0.02M glucose and the rate of respiration or glycolysis was measured in Warburg vessels. In their first publication,[300] alkyldimethylbenzylammonium chloride was found to exert a pronounced inhibitory effect on both respiration and glycolysis of microorganisms associated with dental caries. These included lactobacilli, two strains of *Micrococcus tetragenus, S. albus,* an unidentified aerobic acid-producing Gram-positive diplococcus, and a yeast of the *Monilia* genus. A concentration of the quaternary ammonium compound as low as M/10,000 was found to be effective on 1 billion cells within 5 to 10 minutes. This action was complete and irreversible since it was demonstrated that activity was not regained by washing "treated" centrifugated cell suspension with saline and again testing for metabolic activity in fresh glucose-buffer solution.

In subsequent publications, Miller and his co-workers[218,219,301] described bacterial metabolism studies on a large variety of synthetic detergents. All the quaternary ammoniums (cationics) tested inhibited the metabolism of several Gram-positive and Gram-negative bacteria in a concentration of 1:3,000. Some were also active at 1:30,000 and a few at 1:60,000. The anionic compounds, on the other hand, showed little if any inhibition at a concentration of 1:30,000 and only one was able to completely inhibit the Gram-positive bacteria at a dilution of 1:3,000. In one of the articles,[218] reference was made to unpublished data on the ability of alkyldimethylbenzylammonium chloride to penetrate the dense matrix of human dental plaque material and inhibit the metabolism of the mixed bacterial flora.

Harrison and Miller[301] postulated from their findings that complete absence of bactericidal action of anionic detergents and diminished activity of cationic detergents against Gram-negative organisms may be related to differences in content, type, or cellular arrangement of phospholipids in the Gram-negative as compared to the Gram-positive bacteria. They noted, moreover, when complete inhibition was obtained with the cationic compounds, the action was very rapid, respiration and glycolysis usually ceasing within the first manometric reading of 15 minutes. Furthermore, they were able to demonstrate that alkyldimethylbenzylammonium chloride and *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride, both containing a benzyl group in the nucleus, were more active against bacteria than the cationic detergents with no benzyl group.

In a subsequent publication, Baker, Harrison, and Miller[79] studied the interfering action of phospholipids upon the antibacterial action of synthetic detergents. Based on the assumption that the action of some detergents on bacteria may be due to (1) disorganization of the cell membrane which can be attributed to the surface activity of the agents, and (2) an interaction between detergent and lipoid constituents of the cell membrane as the result of denaturation of proteins, studies were carried out to demonstrate these points. They had previously shown that combination of an anionic and cationic detergent will often result in the formation of a precipitate. Accompanying this incompatibility was the interference of the inhibiting action on bacterial metabolism of the anionic or cationic detergents. However, a different phenomenon appeared to be involved in the protecting action of phospholipids (lecithin, cephalin, sphingomyelin) upon the metabolism of bacterial cells treated with synthetic detergents. They noted that protection with phospholipids was evidenced only when the latter were added before or simultaneously with the detergents. Addition after the detergents failed to show an interfering action by the lipids. A series of vitamins, a yeast extract, methylene blue, glycerol, cholesterol, etc., all proved ineffective against the action of alkyldimethylbenzylammonium chloride upon the metabolism of bacterial cells.

In comparing the activities of quaternary ammonium compounds with several phosphonium and arsonium salts, Jerchel[302] found the former germicide was more effective than the latter two groups of compound in inhibiting glycolysis by lactic acid bacteria. The quaternary ammonium compounds, on the other hand, proved to be less hemolytic to rats' red blood cells than were the phosphonium and arsonium derivatives. The inhibition of glycolysis of lactic acid bacteria, *Streptobacterium plantarum,* by an interesting group of long-chain sulfonamidotetrazolium salts was also demonstrated by Jerchel[50] who used the method described by Baker, Harrison, and Miller[79] for evaluating the quaternary ammonium compounds. Mention may also be made of the observations of Kuhn and Jerchel[303] in which the quaternary ammonium compounds 2,3-diphenyl-5-methyl and 2,3-diphenyl-5-*n*-undecyl-tetrazolium chloride were acted upon by growing bacteria and plants to change the colorless compounds to a distinct red color. The addition of solutions of these derivatives to vegetation (green leaves, for example), results in a change of the chlorophyll to a deep red color.

Ordal and Borg[304] studied the effect of an anionic surface active compound, dioctyl ester of sodium sulfosuccinate, and a cationic agent, cetylpyridinium chloride, on the oxidation of lactate by molecular oxygen as compared with anaerobic oxidation by methylene blue, using *S. aureus* and *E. coli* as the test organisms. From the data given in their findings, it will be noted that the lactate dehydrogenase of *S. aureus* is considerably more susceptible to the action of both anionic and cationic surface-active agents than that of *E. coli*. Both agents inhibited the oxidation of lactate by *S. aureus* but only the quaternary ammonium compound inhibited the oxidation of lactate by *E. coli*.

In tests with *S. aureus*, Ordal and Borg found little difference between the concentrations of the surface-active agents required to inhibit the oxidation of lactate by oxygen, as compared to the oxidation by methylene blue. This fact led them to assume that the cytochrome systems normally involved in the oxidation of lactate by oxygen in *S. aureus* possess no special sensitivity to the surface-active agents. Marked differences in effects of the two compounds were noted on the oxidation of lactate by *E. coli*. Much lower concentrations of cetylpyridinium chloride were required to inhibit the oxidation of lactate by molecular oxygen than by methylene blue. These results indicated to Ordal and Borg that the cytochrome systems of *E. coli* are either more sensitive to the quaternary ammonium compound than the other proteins concerned in the oxidation of lactate, or more readily available to the action of the germicide. Attempts to correlate their findings with those of DuBois and of Baker, *et al.*, are given, with respect to the action of synthetic detergents upon the phospholipid fractions of bacterial cell membranes.

Studies along similar lines were reported by Sevag and Ross[305], who investigated the mechanism of the inhibitory action of alkyldimethylbenzylammonium chloride on yeast cells. They found that concentrations of 1:1,000 or higher of the germicide would cause a marked fading or abolishment of the adsorption bands of a preparation of cytochrome C and of yeast cells. In their manometric measurements a 1:35,000 dilution of the quaternary ammonium compound caused complete inhibition of the oxygen consumption of *p*-phenylenediamine in yeast cells. In the same study, the aerobic and anaerobic oxidation of glucose by yeast cells was also inhibited by the germicide. In the presence of 1.8% serum proteins, a counteraction of these inhibitions was obtained.

In a review on the mechanism of the bactericidal action of various surface-active agents, Hotchkiss[306] presented data and his own interpretation of the effects of the compounds on bacterial cells. The evidence presented up to this time led him to conclude that the enzyme proteins or other vital parts of the bacterial cell must have greater sensitivity to the surface-active agents than do proteins in general. This would include even the other proteins of the bacterial cell itself with which the detergents appear to compete successfully. Hotchkiss was able to demonstrate, moreover, that, when the bacterial cells are cytolyzed, various intercellular substances "seep" out of the organisms, which can be demonstrated by chemical means in the surrounding medium. Growth in nutrient medium can be used to determine whether or not the treated organisms have been simultaneously killed. Evidence was presented to show that whenever the concentrations of surface-active agents were adequate to kill bacteria, leakage of nitrogen and phosphorus out of the cells would also occur. This cytolytic injury was readily demonstrated against *S. aureus, E. coli,* streptococci and yeast cells. Included among the surface-active agents showing these effects were the cationic quaternary ammonium germicides and tyrocidin, the anionic detergents among which were tricresol (*o, m, p*-cresol), hexylresorcinol, and bile salt (taurocholate) and of considerably less marked activity the nonionic detergents. Interestingly enough, the commonly known antiseptics failed to cause a leakage of cellular elements from bacteria. Included in the latter list of germicides were hydrogen peroxide, potassium permanganate, formaldehyde, mercuric chloride, organic mercurials, acridine dyes, iodine, active chlorine, etc.

Evidence presented by Hotchkiss, that treating bacterial cells with tyrocidin or certain detergents results in a leakage into the suspending fluid of nitrogenous and phosphorus-containing compounds prompted Gale and Taylor[307] to study the effects of tyrocidin against *S. faecalis*. Suspensions of the organisms were assayed for internal free lysine and glutamic acid before and after treatment with tyrocidin. These values, as determined by Warburg measurements, were compared with tests made on suspensions of *S. faecalis* in water in the absence of tyrocidin. The latter control suspensions showed no loss in internal free lysine or glutamic acid, whereas the cell suspensions in tyrocidin revealed a complete loss of the amino acids. The cytolytic action of tyrocidin was very rapid. There was, moreover, a quantitative relationship in the amount of tyrocidin added and the number of cells lysed. Gale and Taylor noted that 1.0 mg. of tyrocidin would lyse 10^9 *S. faecalis* cells and 0.1 mg. lysed 10^8 cells. In comparison to these values, 0.1 mg. of tyrocidin would effectively sterilize 10^8 cells per ml. in a broth culture and

0.001 mg. 10^6 cells per ml. in the same medium. Of particular interest to the present review is the statement in their article: "Similar results are obtained with approximately the same concentrations of cetyltrimethylammonium bromide or the dioctyl ester of sodium sulfosuccinic acid.[o] No such effect was noted with gramicidin, sulfathiazole, acriflavine or penicillin." Reference was also made in the publication by Gale and Taylor to a corresponding rupture of the cell membrane by relatively high concentrations of phenol (1% *vs.* 10^6 per ml. cells). This observation may suggest a possible correlation in the mechanism of action of quaternary ammonium compounds and the simple phenol derivative upon microorganisms.

Reference has already been made to the comprehensive studies by Taft and Strandtmann[154,281,282] on the lytic action of alkyldimethylbenzylammonium chloride on invertebrates. Taft[154] observed that against protozoa the action of the quaternary ammonium compound seems to depend in part on the fluidity of the endoplasm and the rigidity of the cortical plasm. As an example, by treating paramecia with a final concentration of 1:100,000 of alkyldimethylbenzylammonium chloride causes the cells to burst within a matter of a few minutes. Several other invertebrates respond to treatments with the quaternary in a similar manner. Rupture of the protozoa, *Tr. foetus,* following treatment with alkyldimethylbenzylammonium chloride, has also been noted by Lawrence.[202]

Kuhn and Bielig[297] studied the effects of a quaternary ammonium compound, lauryldimethylbenzylammonium bromide, against various protein catalases and other enzyme systems, provitamin A of carrots, chloroplastin, albumin, serum, etc. Proteins such as gelatin and egg-albumen were found to be precipitated in a water dilution by the quaternary ("invert soap"), whereas the latter failed to precipitate globulin. This phenomenon was attributed by the authors to the isoelectric point of the proteins. Since proteins are precipitated only in the form of their anions, the negative-charged proteid-ions will react with the positive-charged molecule of the cationic compounds. Kuhn and Bielig suggested these reactions may explain the mechanism of the killing action of quaternary ammonium germicides on bacteria. In other words, the reaction of this group of compounds is with the anion of the cell-protein and the complexes essential for life in the bacterial cell. A similar explanation has been extended for the mechanism of action of quaternary ammonium compounds against plant viruses by Pfankuch and Kausche.[290]

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[o] **See page 198 for trade name and distributor.**

In studying the effects of synthetic detergents and bile salts, Anson[308] noted these agents would denaturate proteins such as hemoglobin and egg albumen at their isoelectric point and keep these treated proteins in solution. Furthermore, if an excess of anionic or cationic detergent is added to bacteria there is denaturation of enzyme proteins, some of which may be necessary for lysis of certain organisms, i.e., pneumococci. Anson, moreover, points out that any proposed study on the poisoning, killing, and lysis of bacteria by detergents should include a whole series of concentrations of the respective agents rather than one concentration in order to determine the actual chain of reactions that may occur in the organism. Schmidt[309] also found alkyldimethylbenzylammonium chloride, and *N*-methyl-(8-oxyquinoliniumdodecylether) methosulfate would precipitate proteins starting at the isoelectric point of the latter and increase lineally with increasing pH until all the protein is precipitated. He noted that, upon the addition of a neutral salt, the pH range of precipitation can be shifted considerably. A comprehensive review on the mechanism of action of synthetic surface-active agents upon bacteria, viruses, enzymes, and proteins in general has been prepared by Valko.[71] This investigator demonstrated that the effective killing concentration of surface-active detergents upon bacteria, in the absence of an electrolyte in a nutrient medium, is considerably higher than in the presence of an electrolyte. The role played by micelles of the surface-active ions and their effects on protein substrates of bacterial cells was also considered.

Fuller[310] described the antibacterial effects of a series of long-chain aliphatic bases of amines, amidines, and guanidines. Among the groups of compounds that were evaluated with respect to their activity against bacteria in broth in the presence and absence of serum were two diquaternary and two monoquaternary ammonium derivatives. In general, the latter two and other groups of bases were considered able to combine, due to their basic nature, with bacterial nucleoproteins and render them inert. Further evidence to support this consideration was the fact that the most active compounds were usually the most toxic and also the more inhibited by serum. The degree of basicity of the several groups of compounds appeared to increase in the following order: di-thioureas, amines, amidines, guanidines, and quaternary ammonium salts. Furthermore, while the latter two strongly basic derivatives were relatively more active against the Gram-negative bacteria, the remaining less basic groups were more active against Gram-positive organisms.

Although surface-active quaternary ammonium germicides were not included in the studies of Burnet and Lush,[311] they proposed a possible mechanism of action of other types of surface-active agents upon viruses. In investigating the virucidal activity of sodium desoxycholate, sodium lauryl sulfate (anionic detergent), saponin, and an antivirus agent from human nasal secretion, they observed a fairly close correlation between the concentrations of the agents capable of hemolyzing rabbit's red cells and the effect against herpes virus. Of the eleven viruses tested, only psittacosis virus was completely insusceptible to the several agents mentioned. No action was observed against dysentery, *Salmonella,* and *S. aureus* bacteriophage. Burnet and Lush postulated from their studies the following: "We might suggest very tentatively that inactivation within the cell is a function of some enzyme capable of disintegrating the surface structure of the virus particle in a similar fashion to that of the surface-active agents we have employed." A similar hypothesis of the possible mechanism of action of surface-active bile salts against virus was extended by Smith[312] who considered the possibility of virus inactivation as being due to the presence of autolytic ferments. He noted, furthermore, the rapidity with which the susceptible pathogenic viruses were completely inactivated by sodium desoxycholate and compared this phenomenon with the bile solubility of pneumococci. This suggested that the virus elementary bodies may actually be lysed by the bile constituents.

A comprehensive discussion on the mode of action of surface-active agents has been presented by Albert.[148] Reference is made to cationic antiseptics as being neutral salts of bases of high molecular weights. Included in this category are listed the basic dyes such as brilliant green, auramine, crystal violet, and the acridine antiseptics. In this same group were alkyldimethylbenzylammonium chloride, cetyltrimethylammonium bromide, and a chlorbenzylate of a higher alkylamide of dimethylamide acetic acid. Albert attributed the action of cationic antiseptics to their basic ions which reacted with the acidic ions of the organisms. Also, these compounds appear to have a greater effect against those organisms that contain an excess of acidic groups (nucleus and enzyme systems) over basic radicals. This may explain the general trend of greater activity of quaternaries against *S. aureus* and other Gram-positive bacteria that contain a high ratio of acidic over basic groups than against Gram-negative organisms where the ratio is reversed. Albert capably used the theory of resonance to explain the striking differences that may be observed in the bactericidal activity of two closely related aminoacridines. In this particular instance reference is made to 1-, 3- and 4-aminoacridine which are bases of about the same activity as acridine and aniline, whereas 2- and 5-aminoacridines are several thousand times as active as the former compounds. Most of the activity of the quaternary ammonium germicide, alkyldimethylbenzylammonium chloride, is due to surface activity. Albert describes the compound as being a two-ended molecule which has a powerful "polar group" bearing NH_2 at one end of its long axis, while the rest of the molecule is comprised of the "nonpolar" group which is made up simply of carbon, hydrogens, or halogens. The longer and heavier these types of compounds are, the greater will be their surface activity.

In studies on the effects of the antibacterial actions of quaternary ammonium compounds, Valko and DuBois[90,313] noted that an active surface-active cation may be markedly diminished in activity by a less active cation even when the compounds contain reasonably comparable chemical constituents in the molecule. As an example, the germicidal action of *N-n*-dodecyl-*N'*-ethylbenzotriazolium ion was found to be reduced considerably in activity in the presence of less toxic salts in the series, i.e., *N-n*-hexadecyl-*N'*-ethylbenzotriazolium ion, etc. They attributed this interference as a phenomenon occurring on the surface of the bacterial cell or within the cell itself. Certain spaces in, or on, the cell may specifically select the harmless cations that protect the organism from the more toxic ions. Valko and DuBois considered it incorrect to assume that the adsorption of surface-active cations upon bacteria is merely a surface phenomenon. They believed surface activity and adsorbability are both dependent on certain structural features of the cations. While these may be equally pronounced in certain toxic quaternary ammonium cations, other types of toxic cations, such as mercuric or dye ions, are strongly adsorbable although they have little surface activity. The protective action for bacteria of nontoxic cations against toxic cations and the reversal of action of toxic cations by high molecular anions were considered to be due to the phenomenon of ionic exchange by bacteria.

Using the Warburg apparatus for studying the metabolic rates of bacteria, Miller, Abrams, Dorfman, and Klein[314] found sublethal concentrations of protamines and histone would increase the susceptibility of Gram-negative bacteria to anionic detergents and tyrothricin. Neither Tergitol 7 nor tyrothricin had any effect on respiration of *E. coli* in the absence of protamine or histone. Low concentrations and proper adjustment of the pH in which the latter two agents were used obviated the possibility that these two substances might exhibit antibacterial actions in the study. Furthermore, no potentiation in activity could be demonstrated with nonselective inhibitors such as merthiolate or phenol.

Weissman and Graf[315] compared the activity of germicidal concentrations of histone and alkyldimethylbenzylammonium chloride upon *B. anthracis* spores. The determinations, made by the manometric technic, indicated that the quaternary was definitely bacteriostatic in concentrations as low as 25 μg. whereas histone sulfate required 400 μg. for complete inhibition. These investigators stated that lecithin inhibited the action of alkyldimethylbenzylammonium chloride against the test organism while little if any antagonistic action of the lipoid was noted against histone activity. In postulating a mechanism of action of the two antibacterial agents against *B. anthracis,* Weissman and Graf correlated their findings with the previous observations of DuBois, Baker, Harrison, and Miller on the interfering action of lipoids on alkyldimethylbenzylammonium chloride to the relatively unstable histone-lecithin complex formed upon addition to bacterial cells. Reference is made in their article to the latter reaction which was earlier described by Henry and Stacy,[316] and by Bartholomew and Umbreit.[317] The latter investigators demonstrated that the ribonucleic acid present in the outer layer of Gram-positive organisms, and in combination with magnesium and cell protein, is responsible for the Gram-stain reaction. Therefore, according to Weissman and Graf, as the histone + lecithin complex is unstable, the former component of this combination starts to react with cellular nucleic acid giving histone + desoxyribonucleic acid, resulting in desoxyribonucleoprotein. Since desoxyribonucleic acid was found to neutralize histone activity, this was further evidence that salts containing these components were formed upon the addition of histone to bacterial suspensions.

Reaction between bacterial cell constituents and a quaternary ammonium germicide, cetylpyridinium chloride, is also suggested from the data of Dyar.[318] He observed that by using the surface-active agent as a mordant, prior to the addition of an aqueous solution of Congo red and with subsequent washing and counterstaining with methylene blue, the cell walls of yeast and bacteria stained red with definite evidence of red precipitate. Furthermore, upon microscopic examination of stained specimens of bacteria, the cytoplasm of the cell appeared to be shrunken away from the cell wall. Dyar, however, does not consider these findings indicative that the quaternary ammonium germicide has not penetrated the cell membrane. This is supported by evidence presented to show that the cytoplasm of vegetative cells in a surrounding medium will stain black when treated with the quaternary salt and a fat-dye, Sudan black B.

Dyar and Ordal[319] studied the electrophoretic mobilities of bacteria treated with anionic and cationic compounds. They noted that treatment with the anionic detergent, sodium tetradecyl sulfate, produce no change, a slight or marked change in the electrophoretic mobility of the test organisms. In other words, if a change occurs, the concentration of the anionic compound at which this first occurs may vary considerably. Using the cationic compound cetylpyridinium chloride results in a general trend of decrease of charge on the cells, reversal of charge, and finally, stabilization of charge. This work represented an attempt to establish a method of characterizing bacterial surfaces.

Kivela, Mallmann, and Churchill[320,321] have more recently studied the electrophoretic mobilities of spores and vegetative forms of bacterial cells before and after centrifugal washing and simple dilutions. They noted that the latter procedures would result in a reversal of surface-active cations without the use of a neutralizing agent. Treatment by the methods given resulted in removal of the positive charge on spores caused by the cation and restoration of the normal charge. Revival of the spores could be also demonstrated by bacteriological cultures. With vegetative organisms, on the other hand, while the normal electrical charge could be restored by washing, the organisms could not be revived. Klein and Kardon[95] also reported that it is impossible to revive bacteria after the latter had been exposed to germicidal concentrations of quaternary ammonium compounds. An account of their findings is given in the following section of this review.

Mallmann, Leavitt, and Joslyn[322] observed that *S. aureus* and *Micrococcus caseolyticus* are more resistant to quaternary ammonium germicides when the organisms are grown in a medium containing fermentable sugars (lactose, glucose, maltose) than when the cultures are grown in a medium lacking the carbohydrates. The Gram-negative bacteria *E. typhosa* and *E. coli,* on the other hand, gave no indication of variation in resistance to quaternary ammonium germicides when grown in the presence or absence of fermentable sugars. No explanation was given for the phenomenon of the variation in response of Gram-positive organisms to quaternary ammonium activity when grown in the two types of media. Lysol, a cresolic type of disinfectant, Benchlophen, a phenolic type, as well as phenol, showed no difference in effect in either medium.

Rahn[91] has demonstrated that quaternary ammonium germicides are strongly adsorbed on the surfaces of organisms. He also postulated that a film is formed on hands submersed in a solution of a quaternary ammonium compound. This film is believed to be oriented with the inactive organic end of the molecule or ions directed toward the skin, whereas the germicidal position of the molecule is toward the outside. This is offered as the explanation for the presence of unharmed organisms on the skin under the "imperceptible" film described by Miller, *et al.*[151] In the studies described by Rahn, approximately 20 ppm. of alkyldimethylbenzylammonium chloride was found to be absorbed by 1 gram of baker's yeast suspended in 100 ml. of 100 ppm. of the quaternary. He noted that when bentonite was added to a solution of the germicide the particles of inert substance increased noticeably in volume, which suggested to him thick adsorption layers. It was inferred from these observations that such layers may retard the diffusion of dissolved molecules to the center of a clump of bacteria.

A somewhat analogous interpretation of the mechanism of interfering action of quaternary ammonium germicides on bacteria may be found in the studies of DuBois and Dibblee.[323] These investigators first treated a suspension of *S. aureus* with an anionic detergent, sodium oleate, before exposing it to a cationic germicide, alkyldimethylbenzylammonium chloride. This pretreatment with the soap resulted in retardation of the action of the quaternary on the organism, which was attributed to the adsorption of the soap on the organism. On the other hand, by substituting a synthetic anionic detergent (sodium dodecyl sulfate) for soap in the test, no such action could be noted. DuBois and Dibblee concluded from their observations, therefore, that prior treatment of bacteria with anionic compounds delays the action of cationic germicides but does not necessarily prevent it.

Neter[324] found that 0.1 mg. of alkyldimethylbenzylammonium chloride would detoxify at least 10 minimum lethal doses of tetanus toxin. This action was independent of any changes that could be attributed to the influence of pH. It was noted also that serum would inhibit the detoxifying action of the quaternary ammonium germicide. The detoxifying action of the compound could not be reversed once it had acted on the tetanus toxin. Neter was unable to state, at that time, whether the quaternary salt merely interfered with the toxic properties of the toxin or also affected its antigenic property.

In a subsequent publication, Neter[325] presented his observations on the effects of alkyldimethylbenzylammonium chloride upon plasma coagulation by staphylococcus and upon fibrinolysis by streptococcus. The germicide, in a dilution of 1:10,000, inhibited and, in dilutions of up to 1:1,000,000, delayed the clotting of oxalated human plasma by staphylococcus cultures. Fibrinolysis by hemolytic streptococcus was inhibited by the germicide in dilutions of up to 1:100,000. Although the exact mechanism of action of the quaternary on plasma coagulation by staphylococcus was not known, Neter postulated that the inhibitory action was more likely directed on the culture or the staphylocoagulase rather than against the mechanism of clot formation.

Holmes[326] studied the effects of anionic and cationic surface tension depressants on serological systems. He noted that the antigen was not affected by sodium lauryl sulfate (anionic) and no permanent change could be evidenced on the bacteria since they were still able to absorb the antibody. The wetting agent also failed to denature antiserum. While alcohols (as surface-active agents) did not alter the course of the reactions in precipitation and agglutination tests, all the soaps studied (oleates) were found to inhibit immunological reactions. Sodium oleate and sodium lauryl sulfate interfered with bacterial agglutination by increasing the negative electrical charge on the organism. Cetylpyridinium bromide (cationic), which acts in an opposite manner to anionic compounds, spontaneously agglutinated the bacteria by markedly lowering their charge. Thus, the quaternary ammonium compounds decrease the stability of the antigens and also cause a nonspecific precipitation of serum.

INACTIVATION OF QUATERNARIES

Reference has already been made in an early section of this review to the compatibilities and incompatibilities that have been noted with various agents and the quaternary ammonium germicides. In regard to certain types of compounds, one can almost predict, on the basis of chemical structure, whether or not the substances will combine bacteriologically as well as chemically with a quaternary ammonium solution. This is particularly true in respect to the anionic synthetic detergents and soaps which in most instances are distinctly incompatible with the cationic detergents.

More recently, however, in order to distinguish between the bactericidal and bacteriostatic activity of these compounds attention has been directed toward providing a suitable "inactivating," "neutralizing," or "antidotal" agent which would counteract the germicidal action of quaternary ammonium salts. As in the case of other effective chemotherapeutic or germicidal agents, studies of this nature not only are directed toward an explanation for the mechanism of action of the new compounds but also serve to aid in determining the presence or numbers of living microorganisms that are inhibited in their development in such solutions.

Examples of methods used to inactivate the antibacterial properties of various compounds may be found in the following: thioglycollate *vs.* mercurials;[327,328] sodium thiosulfate *vs.* chlorine;[329,330] *p*-aminobenzoic acid *vs.* sulfanilamide;[331,332] penicillinase *vs.* penicillin;[333,334,335] ferric chloride or charcoal *vs.* phenol;[336] and glucose *vs.* streptomycin.[337]

In the inactivation of quaternary ammonium germicides, as well as of the antibacterial agents just mentioned, several important factors must be considered in selecting the most desirable agent which is to be used for this purpose. The neutralizing substance must inactivate as completely as possible the bactericidal and bacteriostatic properties of the compound and maintain this state over a reasonable period of time. Moreover, the inactivating agent should have no antibacterial effects itself and little if any growth promoting action upon microorganisms.

Cationic and anionic detergents will neutralize and often precipitate each other. Domagk, who called attention to the high antiseptic properties of the quaternary ammonium germicides, was first to indicate the neutralizing action of an anionic detergent (soap) upon a cationic detergent. Baker, Harrison, and Miller[79] described the inhibiting action of the anionic compounds, decyl sulfate and sodium taurocholate, upon alkyldimethylbenzylammonium chloride. Valko and DuBois[90] found that bacteria which are apparently "killed" by a cationic quaternary ammonium germicide may be revived by the addition of a high molecular anion, provided treatment with the latter occurs within 10 to 30 minutes. Klein and Kardon,[95] on the other hand, have recently demonstrated that "reversal" of quaternary ammonium action cannot be produced once the organisms have been exposed to germicidal concentrations of the compounds. Under certain conditions, however, the addition of an anionic detergent (sodium decyl sulfate, sodium lauryl sulfate, etc.) to the treated bacterial suspensions merely interrupts the continued action of the cationic detergents, thereby permitting surviving organisms to grow out. These authors also found that the action of alkyldimethylbenzylammonium chloride has a selective effect on Gram-positive bacteria which resembles the same bacterial spectrum of penicillin. Evidence was presented to show that the bacteriostatic activity of the quaternary ammonium germicide can be neutralized against Gram-negative bacteria but not against Gram-positive organisms. It is apparent, therefore, that soaps and anionic compounds fail to meet the requirements of suitable quaternary ammonium inactivators due to their inability to fulfill one or more of the prerequisites given. A possible exception is sodium oleate which was considered by James and Eckfeldt[189,190] to be suitable for this purpose and sodium stearate which Pressman and Rhodes[183] used in their studies. In the latter investigation U.S.P. tincture of green soap and a granulate soap were found to be unsuitable as quaternary ammonium inactivators due to the variability in their soap contents. These workers found, however, that the addition of stoichiometric quantities of sodium stearate to the quaternary ammonium test solutions would give the desired inactivating action in their studies.

Reference has already been made of the observations of DuBois and Dibblee[323] in which sodium oleate was found to merely retard the action of a quaternary ammonium germicide against *S. aureus* and not to abolish the activity completely.

Unlike the ionic interaction between anionic and cationic compounds is the interference of the germicidal action of quaternary ammonium compounds by phospholipids. Baker and her co-workers[79] demonstrated the protective action of lecithin, cephalin, and sphingomyelin on bacteria that were subsequently exposed to the cationic germicides. They noted, however, that the lipids were ineffective unless they were added to a suspension of organisms before or at the same time as the quaternary ammonium salts. This factor, therefore, would also exclude the class of lipids as suitable neutralizing agents for the latter group of germicides due to the failure of the inactivator to be effective once the organisms had been exposed to the quaternary ammonium solutions.

The presence of phospholipids or related lipid substances in liver may account for the inactivating action of the liver-broth Zeissler and Günther[269] used for neutralizing the action of alkyldimethylbenzylammonium chloride upon bacterial spores. The presence of lipid substances in oxgall are no doubt the interfering agents responsible for the suppression of the germicidal activity of quaternary ammonium compounds described in the study by Klarmann and Wright.[157]

Several investigators have adopted the use of lecithin in their studies for determining the possible mechanism of action of the quaternary ammonium compounds upon microorganisms. Quisno, Gibby, and Foter[176] described a medium containing lecithin and "Tween 80" (a polyoxyethylene derivative of sorbitan monooleate) for the inactivation of relatively high concentrations of the quaternaries. However, when used alone in the medium, the inactivating agent promoted the growth of some strains of bacteria. Lecithin and Tween have also been used by Stuart[177] and Armbruster and Ridenour[178] in their quaternary investigations.

Lawrence[92,93] tested several anionic detergents, including soaps and a series of compounds known to give a precipitate in the presence of quaternary ammonium solutions. In practically all instances, the compounds studied failed to meet the requirements for a suitable inactivator. Encouraging results were obtained, however, with a naphthalene sulfonic acid derivative, Suramin Sodium, U.S.P. XIII (Bayer 205, Germanin). This compound more closely fulfilled the requirements for an ideal quaternary ammonium inactivator than did any of the other compounds used in the investigation. In itself, it showed no evidence of an adverse action against the test organisms even when allowed to act on the bacteria for relatively long periods of time.

Data were presented in the latter study to show, in the absence of Suramin Sodium, the limiting dilution of a quaternary ammonium germicide that apparently destroyed a dilute suspension of test organism was 1:70,000. In the presence of the inactivator, however, a 1:40,000 concentration of the germicide was required to destroy the same test organisms. Results of studies which substantiate, in part, the findings of Lawrence on the action of Suramin Sodium have been reported by Weber and Black[338] in their investigations on neutralizers for quaternary ammonium compounds used in food utensil sanitation. In addition to the use of Suramin Sodium (Naphuride Sodium) in their studies on the inactivation of alkyldimethylbenzylammonium chloride, tests were made on lauryl alcohol sulfate,[p] lecithin in a polyoxyethylene derivative of sorbitan monooleate and a sulfonated alkyl aryl polyether alcohol. All were found to be effective inhibitors of the quaternary ammonium compound. *E. coli* was used in their studies on swab-rinse plate counts of food utensils sanitized by the germicide. There appeared to be an advantage in using Suramin Sodium or the lecithin-polyoxyethylene derivative of sorbitan monooleate combination as inactivators as compared to the other agents tested. In a subsequent report by Weber and Black[339] preference was given to the use of the lecithin containing medium in the test suspension plus the addition of the same inactivator to the agar in which the quaternary-medication suspension was plated for determining the presence of viable bacteria.

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[p] **See page 198 for trade names and distributors.**

Mueller, Seeley, and Larkin[94] also obtained favorable results with Suramin Sodium in their studies on the evaluation of a quaternary ammonium germicide on dairy equipment. They refer to the necessity of stopping the action of a germicide almost at will in conducting tests to determine germicidal strength. Although bentonite and charcoal were capable of inactivating quaternary ammonium germicides, these agents left their glassware in poor condition. Compounds such as lecithin or phospholipids were not practical since these agents were described as being insoluble in water and, moreover, did not act completely.

Rahn[340] used various combinations of lecithin, charcoal, sodium dodecyl sulfate, and skim milk in his studies on the inactivation of quaternary ammonium solutions. While these "antidotes" or "neutralizers" were found to give varying degrees of interfering action on the germicides, he discouraged the use of antidotes in any standard method for testing disinfectants. This was based on the fact that quickly reacting antidotes are not known for all disinfectants and the modes of action of antidotes upon disinfectants are in many instances also not known. Two possible mechanisms of action of interfering substances on germicides are cited by Rahn. The antidote may remove the excess of disinfectant from the cell surface by adsorption, as in the case with charcoal versus quaternary ammonium compounds and phenol, or they may diffuse in the cell to reverse the reaction between the disinfectant and cell content. An example of the latter is the action of H_2S with $HgCl_2$. In this instance, the cell may actually be revived. Use of charcoal in the former case merely prevents an actual kill from taking place without reversing the reaction where it already has occurred. Rahn concluded that, although the media used in the standard methods do not neutralize or reverse the action of germicides intentionally, the protein or organic matter in the broth reacts materially with the heavy metals, quaternary ammonium compounds, etc., to affect their phenol coefficients greatly.

Goetchius[341] used a sodium salt of a condensed aryl sulfonic acid,[q] as a means for inactivating quaternary ammonium germicides. This compound had been previously demonstrated by Goetchius and his associates[143] to also have a possible application in determining "Use Dilution" concentrations of the quaternary ammonium class of disinfectants. The compound is highly water soluble, will withstand ordinary autoclaving temperatures and is reported to have no bacteriostatic effects on many of the most sensitive bacteria.

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[q] **See page 202 for trade name and distributor.**

7. Pharmacology and Toxicology

Information on the pharmacology and toxicology of most quaternary ammonium germicides on the market today is conspicuous by its absence. The notable exceptions are the publications of Becker and his co-workers,[342,343] and Nelson and Lyster[344] wherein are given comprehensive accounts of the toxicity and pharmacology of cetylpyridinium chloride and myristyl-γ-picolinium chloride, respectively. Becker studied acute and chronic toxicities, following administration of large doses of cetylpyridinium chloride, by the intravenous, intraperitoneal, subcutaneous, and oral routes in mice, rats, guinea pigs, rabbits, and dogs. Intravenous injection of lethal doses (15-20 mg. per kg.) of the quaternary ammonium solution in rabbits resulted in either convulsion or depression prior to death. Death was caused by asphyxia apparently due to paralysis of respiratory muscles. Muscular paralysis of the limbs was noted also in dogs receiving lethal doses of the quaternary ammonium germicide.

Intraperitoneal injection of large doses of cetylpyridinium chloride in rabbits, guinea pigs, rats and mice was observed by Becker to produce greater toxicity than intravenous injection. Of particular significance was the delayed action of the germicide when administered by the intraperitoneal route. No toxic symptoms were noted for from 6 hours to 3 or 4 days, following which the animals exhibited depression and finally died. Autopsy revealed no gross abnormalities nor was there any evidence of inflammation at the site of injection. Subcutaneous injection elicited responses in rabbits comparable to those noted when the intraperitoneal route was used. Death resulting from lethal doses of cetylpyridinium chloride occurred 24 hours to 6 days following injection. Rabbits receiving oral doses of large amounts of cetylpyridinium chloride developed diarrhea. Lethal doses caused death of the animals in from 1 to 6 days. With the exception of the liver and kidney, none of the tissues of dead or sacrificed animals presented any pathological changes.

In irritation studies on cetylpyridinium chloride, Becker and his co-workers found that a concentration of 1:100 or 1:250 produced some reddening when applied to the intact, scarified skin of rabbits. Greater dilutions of the germicide showed no effect in the same tests. Intracutaneous and subcutaneous injections of the quaternary in the dilution recommended for use (1:1000) produced no untoward effect in rabbits. Concentrations of cetylpyridinium chloride of 1:1000 to 1:3000 proved to be definitely irritating to the conjunctival mucosa of rabbits. However, when the corresponding concentrations were injected into the urinary bladder of rabbits there was no indication of irritation or inflammation of the mucous membrane of the animals on autopsy. Studies on the respiratory and cardiovascular systems indicated to Becker and his group that cetylpyridinium chloride exerts a "curare-like" action by producing central stimulation and peripheral paralysis. It is evident from the data presented in the comprehensive study on cetylpyridinium chloride that the 1:1000 dilution, or greater, which is recommended for use, has a wide margin of safety with respect to tissue toxicity or irritation.

Nelson and Lyster[341] studied the local and toxicity effects of myristyl-γ-picolinium chloride in rats, rabbits, and dogs. For purposes of comparison, a limited number of studies were made with cetylpyridinium chloride. The LD_{50}'s for the first quaternary ammonium germicide in rats were 200 mg. per kg. subcutaneously, 7.5 mg. per kg. intraperitoneally, 30 mg. per kg. intravenously, and 250 mg. per kg. by stomach tube. Comparable results were obtained using cetylpyridinium chloride. Following intravenous injection in rats, marked cyanosis and necrosis were observed which in some instances caused loss of the tail at the site of injection. Oral doses of 3% solution caused erosion of the gastric mucosa. As in the observations of Becker and his group on cetylpyridinium chloride, Nelson and Lyster describe the "curare-like" action of fatal doses of myristyl-γ-picolinium chloride with peripheral paralysis and central nervous stimulation. In the chronic toxicity studies in rats, the latter quaternary was fed in the diet of the animals in concentrations as high as 0.1% without interfering with their normal growth rate. However, when a 0.25% solution of the germicide was given as their sole source of fluids, an average loss in weight of 59 grams occurred during the first 10-day period and all the rats were dead before the fifteenth day of the experiment. A 0.1% solution also proved to be toxic and resulted in deaths of the animals, whereas 0.05% and 0.025% solutions permitted average growth rates of 114 and 112 grams.

Application of pads of cotton, saturated with a 3% solution of myristyl-γ-picolinium chloride, to the shaved surface of a rabbit's ear, resulted in damage to the skin as evidenced by inflammation, edema, and scab formation within 72 hours. A 1% solution of the same quaternary, as well as cetylpyridinium chloride, caused only slight scab formation, edema, and inflammation. Nelson and Lyster observed, moreover, that a 1:3,000 solution of myristyl-γ-picolinium chloride produced only a slight irritation in the conjunctival mucous membrane. Concentrations of 1:2000 or greater produced an increasing degree of inflammation. When the germicide was injected into the urinary bladder of rabbits and the animals later sacrificed, examination of the tissue treated with 0.4% solution revealed normal epithelium, submucosa, muscularis, and serosa. There was no evidence of inflammation or other pathological reaction to the drug. Myristyl-γ-picolinium chloride was fed in the diet of dogs in concentrations as high as 20 mg. per kg. without interfering with their growth rate. Histological section of the tissues at autopsy revealed no abnormalities.

Crum Brown, and Fraser,[345] as early as 1868, reported on the results of their investigations on the physiological action, as related to chemical constitution, of various salts of ammonia. Among the compounds tested were several derivatives of tetramethylammonia and trimethylamine. They noted that in frogs tetramethylammonium iodide would produce paralysis and slight muscular spasm. These effects were directly on the cerebrospinal nervous system and striated muscles, respectively. The compound rapidly destroyed the conductivity of the motor nerves by its action on their peripheral terminations. Crum Brown, and Fraser, moreover, considered the changes of physiological action produced by tetramethylammonium iodide in the frog as differing in some respects to the action of strychnine; however, the paralysis of the peripheral terminations of motor nerves is a characteristic effect of the salts of ammonium bases. Although at this early period no consideration was given to the possible antibacterial application of tetramethylammonium or other quaternary ammonium derivatives, it is of significance to note the physiological effect of these compounds on motor nerve endings (as reported by Crum Brown, and Fraser in 1868) was similar to the "curariform" description given to the action of cetylpyridinium chloride in other animals by Becker and Nelson and their co-workers almost a century later.

An early reference to toxicity and irritation of a quaternary ammonium germicide may be found in the publication of Hornung[78] (1935) on alkyldimethylbenzylammonium chloride. Rabbits tolerated oral administration of 3-5 ml. of a 10% solution per kg. body weight; 0.5% of the solution per kg. when administered subcutaneously; and 0.1 ml. per kg. given intravenously. Domagk[20] shortly thereafter reported on the tolerance of rabbits to subcutaneous and intracutaneous injections of 1.2 ml. per kg. of body weight of a 10% solution of the quaternary ammonium compound. The animals tolerated 3-5 ml. per kg. of body weight of a 10% solution given orally. In the same study 3 ml. of cresol or Lysol caused death of the animals within a few hours. Domagk noted, moreover, that guinea pigs tolerated a 1:1000 concentration of alkyldimethylbenzylammonium chloride as their only source of fluid for months without deleterious effect. Administration of the same amount of cresol resulted in having the animals show evidence of diarrhea, followed by death within 1 hour.

Mention may also be made of the observations of Schneider,[87] at approximately the same period, on his toxicity and irritation studies on alkyldimethylbenzylammonium chloride. Subcutaneous injection in rabbits of 0.2 ml. amounts of 1:1,000 to 1:10,000 dilutions of the germicide caused only a slight suggestion of irritation. A 1:100 dilution, however, was found to produce a marked erythematous infiltration in the injected area. Intracutaneous injection with 0.2 ml. amounts of 1:1,000 to 1:10,000 concentrations of the germicide caused but a minimum degree of necrosis. The 1:100 dilution showed marked necrosis in an area of approximately 10 mm. in diameter.

Walter[346] substituted a 1:200 aqueous solution of alkyldimethylbenzylammonium chloride for water as the sole source of liquids for guinea pigs. With the exception of an initial loss in weight, due to failure to drink and to a transient diarrhea, the animals showed no abnormality after three months. Histologic examination of tissue sections at this time also revealed no changes. Walter, furthermore, injected 1 ml. of a 1:1000 or 4 ml. of a 1:1000 aqueous solution of alkyldimethylbenzylammonium chloride intraperitoneally into guinea pigs daily for a period of three months. The animals showed no loss in weight and revealed no gross pathology. Single large doses of 2 ml. per 100-gram body weight caused the death of the animals within 24 hours. Autopsy revealed no gross abnormality other than diffuse peritoneal inflammation. Instillation of a 1:1000 aqueous solution of alkyldimethylbenzylammonium chloride into the conjunctival sacs of human subjects caused burning, stinging, or "scratching" sensations. A 1:5000 dilution gave little if any reaction and the compound in this concentration was considered by Walter to be suitable for effective use as a germicide.

Harshbarger[347] compared the toxicity of several food preserving agents by incorporating the substances in the diets of rats. Inclusion of 0.3% alkyldimethylbenzylammonium chloride in the diets of the animals caused no ill effects. Of particular interest were the following remarks made by Harshbarger on his findings: "In fact, the Zephiran group made slightly greater gain than the control group; and out of seven pairs of rats finishing the trial (beginning when the animals were four weeks old and medicated diet continued for five weeks) five of the rats receiving Zephiran made greater gains than their paired members in the control group." When sodium benzoate was used in the same study, at the rate of 1% in the diet, this salt did not affect the amount of gain. However, when the rate of sodium benzoate was increased to 3%, a definite toxic effect was noted in the animals. On the basis of the alkyldimethylbenzylammonium chloride findings, Harshbarger suggested the possible use of the compound in the control of the bacterial flora of the alimentary canal in studies where these results were desired.

Woodard and Calvery[348] compared the toxicities of nine anionic surface-active agents and one cationic quaternary ammonium compound by oral or intraperitoneal injection in rats and mice. Of the 10 surface-active agents tested, the single quaternary ammonium germicide, alkyldimethylbenzylammonium chloride, was the most toxic to the animals. The LD_{50} dose for rats by the oral route was 0.35 gram per kg. and 0.05 gram per kg. when administered intraperitoneally. The same germicide also proved to be the least tolerated when fed to guinea pigs in that none of 12 animals survived over 40 days when given a 0.2% concentration of the germicide in their water. Of the anionic agents tested, sodium lauryl sulfate appeared to be more toxic. Microscopic examination of the tissues of the guinea pigs showed no damage that could directly be attributed to any of the agents tested, but showed varying degrees of damage typical of inanition.

Fitzhugh and Nelson[349] extended the studies of Woodard and Calvery in their investigations by covering a two-year chronic toxicity study in which the single quaternary ammonium germicide, alkyldimethylbenzylammonium chloride, was compared to several anionic surface-active agents. The former compound was found to be toxic to some rats at a concentration as low as 0.063% (625 ppm.) when incorporated in the diet of the animals. All animals died within 10 weeks when fed 0.5% (5,000 ppm.) of the germicide. A 1% (10,000 ppm.) of sodium dioctyl sulfosuccinate produced a significant retardation of growth of the animals but no histopathological lesions. Sodium lauryl sulfate was not toxic at dosage levels of 1% or less in the diet. However, when the latter anionic detergent was fed for four months in a concentration of 4% (40,000 ppm.) a significant decrease in growth rate was noted. All animals on a diet containing 4% sodium alkyl aryl sulfonate died within a period of four months. The toxic effects of all the surface-active agents studied were produced by irritation of the gastrointestinal tract.

Fitzhugh and Nelson indicated in their study that their investigations were prompted by the fact that surface-active agents were being widely used for cleaning and disinfecting purposes in restaurants, dairies, and other food processing plants. They pointed out that the use of surface-active agents for the purposes mentioned may involve possible ingestion by humans and may result in toxic reactions.

It appears in order to point out that Fitzhugh and Nelson, as well as Woodard and Calvery, used but a single quaternary ammonium germicide and compared the toxicity of this disinfectant to that of five anionic detergents. No reference is made in either publication differentiating the compounds as being anionic or cationic, nor is there any inference to the germicidal activity of the various compounds tested. It is apparent, therefore, that the casual reader of the articles mentioned would unjustifiably question the use of the quaternary ammonium germicide as a disinfecting agent if a comparison of its toxicity was made to that of the anionic detergents such as Duponol, Santomerse, Nacconols, and other sudsing agents found in the many soapless washing powders or shampoos that are on the market today. A further point to be considered is that the lowest concentration of 0.063% or 1:1,600 of quaternary ammonium germicide used by Fitzhugh and Nelson in their feeding experiments is more than double the concentration or 1:4,000 which the quaternary manufacturers recommend for use in the dairy industry and in eating and drinking establishments.

Vivino and Koppanyi[350] incorporated 0.1% of the lauric and myristic acid esters of colaminoformylmethylpyridinium chloride in the drinking water of rats as their sole source of fluids for 25 weeks. The rats were inbred upon reaching maturity and after the young were weaned they also were placed on a 0.1% solution of the germicide. The results of their study revealed that the weight increases in the control and treated groups were comparable. All had normal eating habits and appetites, normal texture of the fur, skin, and other features. Reproductive and muscular activity of the treated groups were normal and no evidence of diarrhea or nausea could be noted. Microscopic examination of the animals that were killed at the termination of the experiment failed to reveal any pathology of the gastrointestinal tract, liver, lungs, kidneys, or bladder.

Teply and Elvehjem[351] added 1:1500 and 1:1200 dilutions of *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride to the drinking water of rats and noted the animals grew poorly on a synthetic ration. However, the addition of 5% whole liver powder to the diet apparently counteracted the quaternary ammonium germicide since good growth was noted upon feeding the liver to the same animals. A combination of folic acid, *p*-aminobenzoic acid, and inositol failed to counteract the effects of the germicide.

Reference has already been made to the studies of Krueger and his associates[292] on the use of alkyldimethylbenzylammonium chloride for the removal of bacterial contaminants from virus suspensions. A 1:1000 concentration of the germicide did not produce pulmonary irritation when given to mice by the intranasal route. Furthermore, 0.1 ml. of a 1:5,000 dilution of the quaternary injected into the allantoic fluid of fertile eggs did not kill the embryos in a period of 72 hours. On the basis of this evidence of low toxicity and certain other features inherent in the compound, Krueger adopted the use of alkyldimethylbenzylammonium chloride in virus studies.[286,292]

Toomey and Takacs[291] compared the toxicity of alkyldimethylbenzylammonium chloride, *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride, cetylpyridinium chloride and cetyltrimethylammonium bromide, in the cotton rat. The animals were given one of several concentrations of the quaternaries by the intranasal, intracerebral, subcutaneous, and intraperitoneal routes. In general, the germicides gave comparable degrees of toxicity. One-half per cent proved to be very toxic and 0.25% killed all the animals within 14 days. The animals were able to tolerate 0.125% in that no deaths occurred within 21 days. It was observed, moreover, that white rats were much more tolerant than cotton rats to the quaternary ammonium germicides. Klein and Stevens[289] injected the first three of the four quaternaries studied by Toomey and Takacs into the peritoneal cavity of mice. They noted that 1 ml. of a 1:1000 dilution of the compounds proved lethal for the animals. No reference was made to the pathological condition of the tissues of deceased animals.

Green and Birkeland[244,245,246] used chick embryos for evaluating the germicidal action of quaternary ammonium and other classes of germicides. In the first of the series of publications, the therapeutic effects of phenol and iodine were compared with cetylpyridinium chloride against experimental *Staphylococcus aureus* infections in the chorio-allantoic membrane of chick embryos. In their preliminary report, the authors presented conclusive evidence that phenol

(1:100) and iodine (1:2,500) had no therapeutic effect, whereas cetylpyridinium chloride had a definite curative action on experimental staphylococcal infections. Survival of many of the embryos treated with the quaternary ammonium compound indicated that a concentration of 1:5,000 was apparently nontoxic to the chicks. In their subsequent publications Green and Birkeland presented evidence on the high therapeutic activity of cetylpyridinium chloride and of other quaternary ammonium salts. Examination of the numbers of eggs injected and the survivals following treatment with the germicides indicated that the embryos apparently tolerated concentrations as high as 1:2,500 of many of the quaternary ammonium compounds.

Paff, Lehman, and Halperin[352] compared the toxicity of several classes of antiseptics on embryonic chick tissue with the action of the compounds on *S. aureus*. They found that the toxicity index (the ratio of the dilution of an antiseptic which will show evidence of toxicity to tissue, to that which will kill a test organism) for phenol and merthiolate was greater than 1, whereas iodine with a value of 0.29 was by far the best drug studied. The single quaternary ammonium compound tested, alkyldimethylbenzylammonium chloride, had an index of the same order as phenol, or 3.4. Saponated solution of cresol U.S.P. XII gave an index less favorable than the quaternary ammonium compound, or 4.1.

Greenwood, Pittenger, Constant, and Ivy[353] studied the effects of alkyldimethylbenzylammonium chloride on the ciliary action of extirpated mucous membranes of normal rabbits. While 1:1000 and 1:2000 aqueous solutions of the germicide, as well as distilled water alone, caused cessation of ciliary action, no apparent deleterious effect on the activity of the cilia could be noted when use was made of the same concentrations of the quaternary prepared in isotonic salt solution (NaCl). In both the saline control and the 1:1000 saline solution of alkyldimethylbenzylammonium chloride, the cilia were beating actively even after 1-hour submersion in the solutions.

Welch and Hunter[354] described a method for evaluating chemical antiseptics in which the germicidal activity of the compounds was compared with their toxicity, against human or guinea-pig leukocytes. In determining the toxicity of several quaternary ammonium germicides Welch, Slocum, and Hunter[355] added citrated human blood to a tube containing an antiseptic dilution and the mixture was rotated for 10 minutes at 37°C. At this point citrated salt solution was added to the suspension to stop further action of the germicide. The mixture was centrifugated for 8 minutes and the supernatant discarded. The layer of phagocytes were tested for activity by the addition of a standardized opsonized *S. aureus* antigen and fresh human plasma. After thorough mixing the degree of phagocytosis was determined by microscopic examination of stained-smear specimens of the test suspension. By comparing the destruction of the phagocytic activity of the blood (*A*) with its toxicity for *S. aureus* (*B*), Vivino and Koppanyi[350] interpreted the findings of Welch to indicate that a wider margin of safety (low toxicity index *A/B*) was obtained on a quaternary ammonium compound (lauric acid ester of colaminoformylmethylpyridinium chloride)-0.5 than that obtained with chloramine T-1.16, crystal violet-1.42, or formaldehyde-30.

Hirsch and Novak[350] also evaluated several germicides, including alkyldimethylbenzylammonium chloride, by their germicidal end points against *S. aureus* in the presence of blood as compared to the toxic end-points which would completely inhibit phagocytosis. Of 24 compounds of diverse chemical constitution, which were available on the market as antiseptics in 1942, only four displayed toxicity indices of less than 1. These compounds listed in their order of preference as based on activity were as follows: alkyldimethylbenzylammonium chloride; mercuric chloride; *N,N′*-dichlorazocarbonamide; and 4-nitro-5-hydroxymercuri-*o*-cresol. In the study, none of the tinctures showed a toxicity index of less than 1. Alcohol 95% and 75% gave an average of 5.2 which the investigators considered responsible for the high unfavorable values obtained on the tinctures.

A review of the literature on the toxicity studies of quaternary ammonium germicides reveals that detailed accounts have been reported on the reactivity of ocular tissues to these compounds. Maier[204] found concentrations of 1:2,500 to 1:4,000 of alkyldimethylbenzylammonium chloride were safe for use in the eyes of rabbits. To demonstrate this point, solutions of the germicide were instilled into the conjunctiva of the animals and kept there for 1 minute. The eyes were washed out with saline and the animals observed for a period of three weeks. At this time the rabbits were sacrificed and the bulbi enucleated, fixed, sectioned, stained, and examined histologically. For comparison a 3.5 concentration of tincture of iodine was used in another animal. One application of the iodine solution resulted in severe inflammation with a discharge of pus. The animals treated with the quaternary ammonium compound, on the other hand, showed little if any reaction to the solution even when treated daily for 10 days. Microscopic examination of the excised tissue revealed no injury to or thickening of the anterior epithelium. The iodine treated cornea, however, showed an enormous thickening, fibrotic changes, and scar formation. On the basis of these findings and the high antibacterial properties of alkyldimethylbenzylammonium chloride, Maier suggested the possible use of the compound for the preservation of biological fluids.

The successful use of the quaternary ammonium compounds for the preparation of patients for surgery, which includes the bacteriology of skin biopsies and irritation or toxicity studies following the use of these germicides, as reported by Keys[357] and others, will be the subject of the following section of this review.

Swan[358] studied the effects of instillation of several anionic detergents, as well as cationic quaternary ammonium solutions, in the eyes of rabbits and human subjects. Strong solutions (0.1%) of both alkyldimethylbenzylammonium chloride and *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride produced a marked and characteristic reaction. Upon instillation of 0.5 ml. into the conjunctival sacs of human volunteers, hyperemia and edema of the conjunctiva developed almost immediately. This was accompanied by a thickening and decrease in transparency of the superficial layers of the conjunctiva which was associated with marked dilation of the superficial capillaries. Other pronounced changes characteristic of the toxicity of the quaternary ammonium germicides was noted. A similar but less severe reaction of the corneal epithelium was seen in the corneas of human subjects, who had been instilling 0.03 to 0.04% of alkyldimethylbenzylammonium chloride solution into their conjunctival sacs three to four times a day for periods of two to eight weeks. In all instances, the conjunctiva and cornea returned to normal within 12 hours of cessation of the use of the drug. Swan extended his studies on alkyldimethylbenzylammonium chloride by injecting 0.025 to 0.050% solution into the interior chamber of the eyes of rabbits. A violent reaction resulted with evidence of the corneal epithelium becoming gray and swollen. In some instances, vesicles were observed to form and rupture. A vivid description of other pathological conditions directly attributed to the toxic action of the quaternary in this form of application was given. In a discussion of his findings, Swan stresses that care should be used to avoid accidental introduction of the quaternary ammonium germicide when used for instrument sterilization, in preoperative preparation, or in treatment of penetrating wounds of the eye. However, the point is also made that concentrations producing irritation in the conjunctival sac are usually greater than the minimal effective therapeutic concentration. Reference is made in the same publication to a personal communication to the effect that a 0.002% alkyldimethylbenzylammonium chloride solution inhibited the respiration of a hemolytic streptococcus of known ocular pathogenicity, whereas a concentration as high as 0.025% was tolerated in the conjunctival sac.

In a bulletin of the U. S. Army Medical Department[359] and a publication by Bellows[360] were presented a series of studies on the effects of antiseptics on the regeneration of corneal epithelium. In the first study, the agents were instilled in the left eyes of rabbits daily, while the right eyes received similar amounts of physiologic saline solution. Among the antiseptics tested in aqueous solution were alkyldimethylbenzylammonium chloride (1:3000), penicillin (2500 units per ml.), and sulfathiazole sodium (2%). Mention was made in both publications to the effect that local antiseptics ordinarily used in ocular therapeutics have considerable delaying action on the healing process of the corneal epithelium, and usually cause permanent opacity. This was presumed to be due to a nonspecific irritation rather than to a specific chemical effect. In the concentrations used, neither penicillin nor sulfathiazole sodium delayed regeneration or produced corneal opacities. The local toxic effects of quaternary ammonium detergents on ocular structure are also reviewed in publications by Irvine[361] and Leopold.[362] The latter author also presented the results of his own observations on the relative toxicity of the various detergents in common use when applied to corneal structures. Confirmation was made of previous published reports on the toxicity to ocular tissues of relatively high concentrations (0.1%) of alkyldimethylbenzylammonium chloride. While repeated application of a 0.1% solution of the quaternary produced conjunctival damage, the changes resulting from a single application of the same or higher concentrations disappeared within 24 hours.

Whitehill[363] determined the maximum nonirritating concentrations of several liquid antiseptics in the eyes of rabbits and guinea pigs. Tests were also carried out to determine the time required for the same dilution of the antiseptic to kill *S. aureus*. The maximum nonirritating concentrations of cetylpyridinium chloride and a refined grade of alkyldimethylbenzylammonium chloride in the eyes of the test animals were 1:2000 and 1:3000, respectively. Both quaternaries destroyed the staphylococci in one-half minute at 37°C. Sodium ethylmercurithiosalicylate, in the same study, was nonirritating in a concentration of 1:1000, but required 60 minutes to destroy *S. aureus*. With phenyl mercuric nitrate the nonirritating concentration and killing time were 1:10,000 and 22½ minutes respectively. With silver nitrate these values were 1:300 and 45 minutes. Whitehill concluded from his observations that the organic mercury compounds were the poorest agents he studied. The killing action of these compounds for bacteria was so slow that they were considered to be of value only in forms of wet packs or when incorporated in ointments.

Thompson, Isaacs, and Khorazo[364] evaluated a number of antiseptics with reference to their use in ophthalmology. A number of factors were enumerated with respect to the theoretical and practical consideration of the "Law of Disinfection," i.e., kind of organism, degree of dispersion (clumping), presence or absence of organic matter, pH, and temperature of the medium. Alkyldimethylbenzylammonium chloride was included in the list of 10 antiseptics commonly used for ocular application. A 0.15% concentration of the quaternary ammonium germicide proved to be irritating to the eyes of rabbits, whereas little if any inflammation was observed when a 0.075% concentration was used. In comparison to these observations, acriflavine showed a toxic reaction in 0.25% as well as 0.10% concentrations; gentian violet in 0.05% sodium ethylmercurithiosalicylate in 0.04% and 0.125%; phenylmercuric nitrate 0.02 and 0.01%, etc. Thompson and his co-workers used the nonirritating concentrations of the several antiseptics and determined their disinfecting action against *S. aureus*. The medium used in this study simulated tears and consisted of 10% serum in physiological saline solution. Their data reveal that iodine and silver nitrate are markedly reduced in bactericidal activity by the presence of organic matter. The most rapidly acting germicide tested was the quaternary ammonium compound, alkyldimethylbenzylammonium chloride. A concentration of 0.04%, which was the maximum nonirritating concentration, destroyed the test organisms in a matter of 3 seconds. Complete sterilization was not obtained with any of the other antiseptics even after 10 minutes. Reference is made to the possible interfering action of the quaternary on the bactericidal effects of normal blood and lysozyme action.

With reference to the use of surface-active compounds in ointment bases, Dodd, Hartmann, and Ward[365] used 9 surface-active agents as the major components of hydrophilic ointment bases. These were tested in the eyes and on the skin of normal rabbits. Only mannitan monooleate, a polyoxyalkylene derivative of sorbitan monostearate, and the glycerin ester of lauric acid showed little if any evidence of irritation in preliminary studies. Several ionic agents, among which were two cationic compounds, cetyldimethylbenzylammonium chloride and the stearylamine salt of the stearylamide of ethyl phosphoric acid, were found to behave as chemical irritants. On the skin of rabbits these compounds caused local edema, necrosis, and hyperemia. They also acted as primary chemical irritants on the skin of human subjects. For the reasons given, these and other ionic agents tested were not considered suitable for inclusion as components of ointment vehicles.

A publication by Draize, Woodard, and Calvery[366] presented quantitative and qualitative methods for obtaining information concerning compounds intended for topical application to the skin and mucous membranes. In the latter respect, irritation of the mucous membranes was measured on the rabbit eye and penis. Application of a 0.2% solution of alkyldimethylbenzylammonium chloride to the penal mucosa of the animals resulted only in a very slight irritation which was noted after 1 to 2 hours. In a strength of 2% solution, irritation was moderate after 24 hours, but continued to a maximum reaction after 24 hours. In the same tests a 2% solution of dioctyl sodium sulfosuccinate proved to be equally as irritating to the mucous membrane as the quaternary ammonium solution at the 1- to 2-hour observation period. However, the degree of irritation did not become accentuated, as with the quaternary ammonium germicide, when examined 24 hours later.

Barail[367] reported on the toxicity of several mercurial germicides and alkyldimethylbenzylammonium chloride. Since he was able to demonstrate that the quaternary ammonium compound was nonirritating to the skin of animals but caused a definite irritation on human skin during patch tests, he cautioned against the use of these compounds for disinfecting and sterilizing infants' clothing.

An analysis of the data presented by Barail, as well as that of Hoyt, Fisk, and Burde,[368] and Sprague[369] on the acute toxicity of alkyldimethylbenzylammonium chloride when injected intraperitoneally into mice, reveals that the LD_{50} dose of the germicide is approximately 20 mg. per kg. It is, therefore, not surprising to note that an 18-20 mg. mouse that received 2½ times the latter dose died within a short time as reported by Barail. Sprague has demonstrated, moreover, that the intradermal or subcutaneous injection of a 1:1,000 aqueous solution of alkyldimethylbenzylammonium chloride in amounts of 0.1 ml. causes little or no irritation at the site of injection. This same concentration applied on a patch test under a rubber dam to the clipped abdomen of rabbits for 8 hours produces but a mild erythema within 72 hours.

Ordal and Rucker[228] used several quaternary ammonium compounds as a means for controlling gill disease in fish. They cautioned, however, to the relative narrow margin of safety between the effective therapeutic concentrations and the concentrations that are toxic to fish. A comparison was also made of the lethal effects of a homologous series of alkyldimethylbenzylammonium chlorides against *Chondrococcus columnaris,* a myxobacterium causing gill disease in fish, as compared to the toxicity of the derivatives to fish. Their results indicated that the dodecyl and tetradecyl homologues were relatively more effective when the above two factors were taken under consideration. Ordal and Rucker demonstrated, moreover, that the germicidal action of certain homologues in the series was enhanced by the presence of organic materials containing nitrogen.

8. Practical Application—Surgical

PREOPERATIVE GERMICIDE

Wetzel[370] as early as 1935 reported on the use of alkyldimethylbenzylammonium chloride as a surgical hand-disinfecting solution, comparing its activity with that of 60% alcohol. The surgeon's hands were brushed in soap and hot water for 10 minutes and then rinsed in a germicidal solution for 5 minutes. Wetzel found that a 1:1000 dilution of the quaternary ammonium compound was superior to alcohol in the disinfection of hands. In further tests, the surgeon's hands were prepared as described and, while they were still wet with the germicide, were covered with surgical gloves. Following the operation, the gloves were removed and sterile saline poured over the hands. Samples of the saline were plated in nutrient agar and a count made of the colonies that subsequently developed in the medium. In many instances, the quaternary-treated hands were found to be sterile. The author concluded from these studies that alkyldimethylbenzylammonium chloride was far better than alcohol as a hand-disinfecting agent.

In describing his clinical experiences with the use of alkyldimethylbenzylammonium chloride, Schmidt[371] also considered that the inherent features, i.e., odorlessness, low toxicity, and colorlessness of the germicide, favored its use over other available germicides in obstetrics and gynecology. Application of solutions of the quaternary ammonium compound to surgical gloves facilitated the ease with which they could be drawn on the hands. The germicide was also used by Schmidt for the sterilization of instruments and in the treatment of certain gynecological conditions.

Caesar,[372] Seemen,[81] and later Nagel[215] also reported on the advantages of the use of the quaternary ammonium germicide over other disinfectants for the disinfection of hands in surgical washes. Mention was made by these investigators of the high germicidal action of alkyldimethylbenzylammonium chloride with minimal or complete lack of irritation to the skin. They reported also on the use of the compound for the treatment of wounds and the cold sterilization of instruments.

Jotten and Schon[373] found that a 1:2,000 solution of alkyldimethylbenzylammonium chloride would destroy *E. coli* organisms that were experimentally placed on hands and the culture permitted to dry thereon. The concentration given would destroy the test organisms within a period of 1 minute, whereas, a 1:2,500 solution required 3 minutes to kill *E. coli* bacteria. They also noted that the hands would remain sterile for a period of at least 1 hour when treated with the quaternary ammonium solution and then protected with rubber gloves. Alkyldimethylbenzylammonium chloride was considered superior to mercuric chloride as a hand disinfectant by these investigators. Hettche[88] also found that alkyldimethylbenzylammonium chloride as well as a chlorbenzylate of a higher alkylamide of dimethylaminoacetic acid would effectively destroy *E. coli* and *S. aureus in vitro* in less than 2 minutes. One per cent solutions of both germicides were as effective as 5% cresol in their destructive action against the organisms mentioned. Based on these observations, the quaternary ammonium compounds were advocated for use as hand-disinfecting agents and for the sterilization of surgical instruments.

Neufeld, Schiemann, and Kuhn,[89] and later Gottsacker[374] found that alkyldimethylbenzylammonium chloride and the chlorbenzylate of a higher alkylamide of dimethylaminoacetic acid were effective in killing pathogenic bacteria *in vitro* as evidenced by failure of the treated organisms to develop in agar or to cause the death of experimentally infected animals. However, in conducting hand-wash experiments somewhat different and unexpected findings were noted when the compounds were used as skin-disinfecting agents. In tests with Type I pneumococci a 1:32,000 dilution of alkyldimethylbenzylammonium chloride was found to destroy the organisms *in vitro.* Three drops of the same culture was diluted 1:10 in broth and rubbed on the tips of fingers. The suspension was allowed to dry for 3 minutes and the fingers then dipped in nutrient medium. The fingers were next washed for 2 minutes in the germicide, following which the latter was rinsed in warm running water. The hands were dried and the fingers dipped in 10 ml. of nutrient broth for 45 seconds. One milliliter and 0.1 ml. of the test broth was then injected intraperitoneally into mice. Treatment of the fingers with a 1:1000 solution of alkyldimethylbenzylammonium chloride resulted in 18 survivals of 39 animals injected with the undiluted test broth culture and 22 of 24 survivals when injected with the 10^{-1} dose. Results comparable to the latter were obtained when the fingers were treated with 1:1000 of the quaternary ammonium compound and then finally dipped in rabbit serum instead of nutrient broth. Neufeld and his co-workers concluded from their studies that treating the hands with a quaternary ammonium solution, with or without a previous wash in soap or water, will not result in complete sterilization of the hands of virulent bacteria (pneumococci). This may be evidenced by the fact that there are a sufficient number of viable organisms remaining on the hands after this treatment to kill at least one of ten experimental animals. The authors commented on the report of Hettche by referring to their inability to confirm his findings that alkyldimethylbenzylammonium chloride and the chlorbenzylate of a higher alkylamide of dimethylaminoacetic acid were highly effective as hand-disinfecting solutions.

The first informative and detailed account of the use of alkyldimethylbenzylammonium chloride in surgery in the United States appeared in the publication by Walter[346] in 1938. Favorable skin tolerance was obtained in 2000 cases in which a 1:1000 dilution of the quaternary was used as the preoperative skin disinfectant. The skin of every anatomic region and the mucous membranes of all the orifices of the body were exposed to the germicidal solution. Lack of irritation of the skin of the hands and arms, following prolonged and repeated use of alkyldimethylbenzylammonium chloride, prompted Walter to study the use of a 1:1000 tincture of the germicide as an arm soak. One hundred and thirty-six individuals participated in this investigation. The solution was used by the group on a total of 10,000 occasions during an 18-month period without evidence of immediate irritation. The same group also used a 1:5000 aqueous solution of the germicide in glove basins. The compound facilitated application of the gloves to the fingers and after its use the skin was not irritated but was "satiny" smooth. The scalps of three patients were scrubbed with a 10% aqueous solution of alkyldimethylbenzylammonium chloride and the moistened hair, encrusted dirt, and blood shaved off. In no instance was there any evidence of skin irritation. In order to determine the bactericidal efficiency of alkyldimethylbenzylammonium chloride, tests were made on full-thickness skin biopsies of 75 patients. The specimens were first washed with Ringer's solution to remove the germicide and then placed in nutrient broth. Seventy-three per cent of the biopsies were sterile. Of the 25% positive cultures, staphylococci were identified in 21, Gram-positive bacilli in 3, and streptococci in 1.

Barnes,[375] Williams, *et al.*,[214] and Gardner and Seddon[376] reported on the use of relatively high concentrations (1%) of cetyltrimethylammonium bromide in cleaning unwashed skin. The compound was advocated for use in cleansing of accidental wounds. In addition to its effectiveness as a germicidal agent, it was found to be more active than soap in removing dirt, congealed blood, oil, grease, and tar from the injured area. Note was made of the fact, however, that repeated application of cetyltrimethylammonium bromide may result in mild degrees of toxicity to the skin, and also that some individuals may be sensitive to the compound. Forman[377] found 2% solutions of cetyltrimethylammonium bromide to be highly effective in the treatment of impetigo. In addition to its superiority to soap in cleansing the skin of ointments, it facilitated removal of scabs and crusts from impetiginized areas or where there was widespread dermatitis or eczema. Satisfactory results were obtained in cases of severe nummular eczema, impetigo of the face, and generalized boils. Trauma, which is associated with the use of soap and water washes, was not noted in the quaternary-treated individuals. Cetyltrimethylammonium bromide has also been used successfully in a cream in combination with a sulfa drug for the treatment of burns.[378] The addition of 1% of the quaternary ammonium compound to a cream was found to facilitate the removal of most of the detachable organic material from a burned surface and to promote the destruction of bacteria. These actions were attributed to the powerful detergent properties inherent in cetyltrimethylammonium bromide.

While the English workers (Barnes, Williams, and Forman) had reported on the successful use of cetyltrimethylammonium bromide in washing unclean skin surfaces, including wounds, through the period 1942-1943, the first reference to the use of the compound in medical practice in the United States was made by Toomey, Kohn, and Lohrey in 1945.[379] These investigators used the compound in a series of 102 cases of impetigo, ringworm, herpes, and miscellaneous cutaneous infections. They concluded from their studies that the quaternary ammonium germicide was of definite value in impetigo but less useful in ringworm or herpes. Fischer[380] used a lotion containing 0.16% cetyltrimethylammonium bromide on 3,520 consecutive patients admitted to a hospital. No cases of impetigo developed following the use of the preparation. Furthermore, no irritation resulting from the use of the lotion was noted in 4,000 infants and children.

Hagan *et al.*[381] compared the activity of cetylpyridinium chloride, alkyldimethylbenzylammonium chloride, mercurials and cresolic-mercurial compounds used as cutaneous germicides on 575 patients prepared for major surgery. The two quaternary ammonium compounds gave comparable degrees of germicidal action which surpassed that noted when the other types of germicides were used. Hagan and his co-workers were also able to demonstrate that the antiseptics with detergent or wetting action retained their effectiveness throughout operative procedures for longer periods than did mercurials and cresolic-mercurial compounds.

In a clinical appraisal of cetylpyridinium chloride as a skin antiseptic, Helmsworth and Hoxworth[382] found that the skin of 247 patients readily tolerated the germicide. On the basis of their investigation, the use of the quaternary ammonium compound was deemed preferable, as regards physical characteristics, economy, and efficacy, to the regular preoperative procedure of scrubbing with soap and water, alcohol, and ether. Abramowitz[383] presented a review of the literature on dermatology and syphilology in which wetting agents, including the quaternary ammonium compounds, were mentioned as being superior in many respects to iodine, alcohol, and other antiseptics. Alkyldimethylbenzylammonium chloride was described as causing a rapid degermination of the skin.

Lovell[384] used a 1:1000 aqueous solution and a 1:1000 tincture (50% alcohol, 10% acetone) of alkyldimethylbenzylammonium chloride for preoperative preparation of the hands. His studies revealed that both solutions were better chemical cleansing agents than 70% alcohol. Soap, however, proved to be a better detergent for the mechanical cleansing of the hands and arms than the 1:1000 quaternary solutions. The best results were obtained when soap was used as the detergent for mechanical cleansing and a 1:1000 aqueous solution of alkyldimethylbenzylammonium chloride as the chemical cleansing agent. Lovell found that scrubbing with soap and water between clean operations was not necessary, but that flushing the hands and arms in running water to remove the powder and perspiration and then washing in a 1:1000 solution of aqueous alkyldimethylbenzylammonium chloride for 3 minutes was sufficient.

White, Collins, and Newman[270] prepared operative sites for surgery with soap and water, benzene, alcohol, and alcohol-acetone tincture (1:1000) of alkyldimethylbenzylammonium chloride. Only a single wound infection occurred in 51 cases prepared by the procedure given, whereas there were 7 wound infections (28%) in 25 cases in which 3.5% tincture of iodine was used for preparing the skin. Biopsies of the skin taken before and after the first cleanup of 51 cases revealed that cultures could be obtained in all instances before the skin preparation and in only two instances from biopsies taken after the skin preparation. Poppe[385] found that 76% of the basins of sterile water used for washing surgical instruments and the hands of operators during the course of operations were contaminated at the close of operations. The presence of 1:5000 alkyldimethylbenzylammonium chloride in the basins resulted in a sterile solution throughout and after the operative procedures.

Schumaker and Bethea[386] made an extensive study of the use of alkyldimethylbenzylammonium chloride in the surgical and laboratory services of the 118th General Hospital and concluded that a brief soaking in a 1:1000 aqueous solution prior to the donning of surgical gloves would bring about as good a preparation of the hands as the most vigorous soap and water scrubbing followed by soaking in 70% alcohol. On the basis of these experiments, the compound was used routinely in presurgical scrub procedures. Alkyldimethylbenzylammonium chloride was also found to be more effective than soap and water in removing oil and greasy débris. It did not cause skin irritation even when used daily for extended periods of time, and no untoward effects were noticed when the recommended concentrations of the germicide accidentally ran into the eyes, ears, nose, and mouth of patients. Furthermore, no tissue reaction of retardation of healing was observed when the solution was poured in wounds. Use of suitable concentrations of the quaternary ammonium compound was also recommended for the sterilization of surgical instruments, for compresses and soaks for pyogenic lesions, and in irrigating empyemas, abscess cavities, urethral instillation, fungus infections, etc.

Kramer and Sedwitz[387] subjected cetylpyridinium to a large series of clinical and bacteriological studies in a hospital. A 1:200 alcohol-acetone tincture of the quaternary was used routinely in 18,000 operative cases without any evidence of irritation or infection. Complete bacteriological studies on 50 surgical patients showed that the

1:200 solution of the germicide was highly effective in producing a sterile operative field. Kramer and Sedwitz found, moreover, that a 1:1000 aqueous solution of cetylpyridinium chloride served as an ideal germicidal irrigant or wet dressing because of its combination of high germicidal activity and detergency.

Indications for the use of alkyldimethylbenzylammonium chloride for the preparation of operative sites for surgery or anesthesia may also be found in the publications of Cutler and Zollinger[388] who used 70% alcohol followed by a 1:1000 solution of the germicide in preparing patients for total thyroidectomy. Hanley and Malone,[389] Brown,[390] Northrup,[391] and Turell,[392] used the solution for preparing areas for caudal anesthesia. Dickerson[393] employed a 70% alcohol solution (tincture) of alkyldimethylbenzylammonium chloride following preliminary cleansing with green soap and water in preparing patients for laparotomy. Clagett, Payne, and Moersh[394] included a 1:10,000 aqueous solution of the quaternary ammonium compound in their regime for preparing a site for operative intervention of tuberculous esophagotracheobronchial fistula. Lape[395] used alternating scrubs of alcohol and the germicide for preparing operative sites for surgery of chronically infected ingrown toenails. Hubly, Mustard, and Stiefel[396] utilized alkyldimethylbenzylammonium chloride as the sterilizing agent for closing pilonidal cysts following surgical excision of the latter. Ingraham, Matson, and Woods[397] described a simple method of reducing the pain of hypodermic injections by the local application of cold. Alkyldimethylbenzylammonium chloride or alcohol was used to prepare the skin area for injection.

Hufnagel, Walter, and Howard[398] have recently described an *in vivo* method for evaluation of detergents and germicides in which multiple determinations can be made of the bacterial flora of the human skin under standardized mechanical scrubbing conditions. Among the various agents tested for their ability to reduce the bacterial population of normal skin were coconut oil soap; a detergent cream consisting of an emulsion of petrolatum, lanolin cholesterols, and sodium *p*-tertiaryoctylphenoxyethoxyethyl ether sulfonate; a mixture of the latter emulsion and a 2,2′-dihydroxy-3,5,6-3′,5′,6′ hexachlorodiphenylmethane; and alkyldimethylbenzylammonium chloride.[r] The curves representing the reduction in bacterial counts following ten scrubs with coconut oil soap and the detergent cream were extremely similar. Both gave an initial count of 1700, and after scrubbing, a count of approximately 10. The latter value was in contrast to the control of 244 bacteria. In the alkyldimethylbenzylammonium chloride series of tests the reduction in average number of organisms was much more rapid from an initial count of 2,500 to 130 organisms during the first 10 strokes. The total average number of organisms after use of the various agents for 30 seconds and ten strokes were as follows: alkyldimethylbenzylammonium chloride, 237; coconut oil soap, 1,075; detergent cream, 1,207; water control, 5,172. A combination of the detergent cream and 3% of the chloromethane compound gave even more striking results with an initial count of 1,500 which was reduced to seven colonies after the first 10 strokes.

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[r] **See page 198 for trade names and distributors.**

STERILIZATION OF INSTRUMENTS

Reference has already been made in the preceding section of this review to the early use of alkyldimethylbenzylammonium chloride by Wetzel;[370] Schmidt,[371] and others, for the sterilization of surgical instruments. Hornung[78] was probably the first to recommend the addition of sodium carbonate to the quaternary ammonium solutions as a means of rust prevention of instruments that were to be stored in the germicide.

Caesar[372] recommended that a concentration of 1:100 alkyldimethylbenzylammonium chloride be used for the disinfection of instruments. He specified a time period of at least 20 minutes to be allowed for proper sterilization following which the instruments are removed from the solution and dried with a towel. The instruments were again to be placed in the sterilizing solution for 5 minutes just prior to use.

In replying to an inquiry of a surgeon in regard to a suitable substitute for bichloride of mercury in hand disinfection and in the sterilization of instruments, Seemen[81] in 1936 suggested the use of alkyldimethylbenzylammonium chloride. For the disinfection of instruments, a 1:100 dilution of the germicide was proposed, and also the use of sodium carbonate in the solution was mentioned as an antirust agent. Walter[346] considered a 1:1000 aqueous solution of alkyldimethylbenzylammonium chloride to be sufficiently active for the sterilization of instruments provided a period of 30 minutes was used in the treatment. He cautioned, however, that the instruments should not be stored in the germicide. Although reasons for the latter point were not given, one may infer that the solution would corrode the instruments. The use of an antirust agent was not considered in this publication.

Winkler and Redecker[80] were apparently the first to actually investigate the disinfecting properties of a quaternary ammonium compound on infected instruments. In their studies the value of alkyldimethylbenzylammonium chloride as a disinfectant was determined in experiments on infected dental instruments. Dental burs were contaminated with a broth culture of *S. aureus* and, after the organisms were permitted to dry for 1 hour, the burs were submersed in an aqueous solution of the quaternary ammonium solution. At varying time intervals, the burs were removed from the germicide, rinsed in distilled water, and placed in a nutrient medium. Incubation of the latter was carried out at 37°C. for four days and the presence or absence of growth recorded. In a similar manner burs were contaminated with a blood suspension of *S. aureus*. Winkler and Redecker found a 1% aqueous solution of alkyldimethylbenzylammonium chloride would destroy all the organisms in the absence of blood in 1 minute. In the presence of dried blood, a concentration of 2% of the quaternary was required to destroy all the test organisms. Upon extending the time of contact of the germicide with the instruments from 1 to 10 minutes in the same procedure alkyldimethylbenzylammonium chloride was found to destroy *S. aureus* in the dried broth culture and in the dried blood in concentrations of 0.1% and 0.3%, respectively. A 0.5% concentration destroyed the same organism in blood in 5 minutes and in broth in 2½ minutes.

Instruments were also contaminated with *B. subtilis* and the latter dried at 37°C. Sterilization of the spore-forming organism was accomplished by a 0.5% concentration of alkyldimethylbenzylammonium chloride in 45 minutes and by a 1% concentration in 30 minutes. Instruments were also contaminated with organisms contained in pus obtained from gangrenous root canals. The bacteria included a greening streptococci, nonhemolytic streptococci, *E. coli* and *B. subtilis*. *E. coli* and the streptococci on instruments were destroyed by a 0.5% solution of the quaternary ammonium germicide in 5 minutes and in 2½ minutes by a 1% solution. *B. subtilis* in the same study were destroyed by a 1% solution between 15-20 minutes. They recommended the use of a 1:20 dilution of the germicide to which is added 50% glycerine. This solution proved to be equally useful for disinfecting and lubricating the instruments. Probably the first suggestion for the use of sodium nitrite as an antirust agent appeared in the article by Winkler and Redecker.

Post[88,399] considered alkyldimethylbenzylammonium chloride to be one of the most extensively studied and most satisfactory of 2000 or more synthetic detergents as a substitute for boiling water in the sterilization of instruments. He noted that a 1:3000 dilution of the germicide proved to be most effective. Reference was made to the effect that no deleterious action on the eyeball was noted even though a concentration of 1:300 of the solution was used by mistake in the surgery for one month. The 1:3000 concentration was found to be sterile when exposed to the air for 5 hours. Use of 0.5% sodium nitrite was recommended as an antirust agent in the solution. In the experiences of Post, approximately 600 intraocular operations were performed with only one instance of infection. The latter was attributed to the failure of dipping the instruments in the germicidal solution immediately before use.

Brief mention may be made of an editorial[400] on the chemical sterilization of instruments. Reference is made to the limitations of this means of sterilization which cannot be relied upon as a safe technic. In regard to quaternary compounds the point is brought out that they lose their bactericidal potency in the presence of soap, protein, and other types of detergents (anionic). Within these limitations, however, the quaternary ammonium compounds were acknowledged to have received favorable acceptance by many as cold disinfectants for medical and dental instruments.

Schumaker and Bethea[386] contaminated surgical instruments with broth cultures of *S. aureus, S. pyogenes, B. proteus, E. coli,* and *Cl. tetani.* Immersion in a 1:1000 aqueous solution of alkyldimethylbenzylammonium chloride completely destroyed all the test organisms in 5 minutes. A 1:2500 solution of the germicide did not give absolute sterility even after 15-minute exposure. They also recommended the use of an antirust agent in conjunction with the use of the quaternary ammonium compound.

Lundy *et al.*[401] used 1:1000 solutions of *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride or alkyldimethylbenzylammonium chloride for the cold sterilization of plastic intratracheal tubes. Use of the quaternary ammonium solutions was made following preliminary washing of the tubes with soap and water. Nicholson and Eversole[402] suggested the use of a 1:1000 aqueous solution of alkyldimethylbenzylammonium chloride for sterilizing ampules containing spinal anesthetics as one means of avoiding neurologic complication of spinal anesthesia. The addition of a dye such as methylene blue to the solution in which the ampules were immersed served as a means of detecting defective ampules. They, furthermore, warned against the use of irritating solutions containing alcohol, phenol, formaldehyde, etc.

WOUND IRRIGATION

Hochmuth[403] as early as 1936 used alkyldimethylbenzylammonium chloride for the irrigation of wounds. Samples of the solution taken after use indicated a 1:3000 dilution of the germicide to be sterile. Preference was given to the use of the quaternary ammonium germicide over potassium permanganate as an irrigating fluid in surgical procedures. Wright and Wilkinson[404] evaluated 1:1000 and 1:5000 aqueous and the 1:1000 alcohol-acetone tincture of alkyldimethylbenzylammonium chloride in the treatment of wounds and skin surfaces. Eighty cases of deep and superficial lacerations of the scalp, face, neck, and extremities were selected for this study. The tincture was used in wounds and skin surfaces, whereas the aqueous solutions were selected for deep lacerations. In some instances the wounds were sutured and in others they were approximated with adhesive. In only one case did infection occur and this eventually cleared after additional treatment with a quaternary ammonium solution. Excellent results were also obtained by the use of the germicide in other surgical cases. On the basis of their findings, Wright and Wilkinson recommended the following scheme of "Use Dilutions" of alkyldimethylbenzylammonium chloride: (1) disinfection of wounds, 1:1000 tincture; (2) deep lacerations, 1:5000 aqueous; (3) wet dressings and irrigations, 1:5000 aqueous; (4) preoperative scrubs, 1:1000 aqueous or tincture.

In the treatment of unusual fractures of the forearm, Hauser[405] used a special technic which consisted of washing the wound surfaces and skin edges with a 1:3000 dilution of alkyldimethylbenzylammonium chloride for 5 minutes. During the removal of foreign bodies repeated washings with the germicidal solution were made. In one of the two cases, the protruding bone ends and muscles were thoroughly washed and irrigated during the operative procedure. These procedures were considered to be contributing factors in the prevention of the development of bacteria and safeguarded against reinfection in the two unusual fractures treated.

Poth, Barnes, and Ross[406] used a 1:4000 solution of alkyldimethylbenzylammonium chloride following daily preoperative baths in the successful clearing of a leg of numerous furuncles. Following the operation for elephantiasis, the skin grafts were dressed with glycerine containing a 1:1000 solution of alkyldimethylbenzylammonium chloride. Brief mention has also been made by Arden[407] of the use of a 1:1000 aqueous solution of the latter germicide in conjunction with 5-aminoacridine for the treatment of wound infections.

Gross[408] reported six cases of the rare syndrome of arterial embolism and gangrene in infants. Precautions were cited for the prevention of secondary infection after gangrene has set in. Recommendations were given for the cleansing of the affected part by a bland antiseptic such as 1:1000 aqueous solution of alkyldimethylbenzylammonium chloride. In two of the six cases where sloughing occurred, healing was rapid and uncomplicated due to the maintenance of sterile precautions.

Stitt[409] described his experiences in the use of organic mercurials, phenols, quinolines, and quaternary ammonium germicides which have been advocated as bronchial lavages in the treatment of nontuberculous infections. In listing the prerequisites that are desired of a germicide of choice for this purpose, Stitt notes that the agent must be relatively nontoxic and should, in well-tolerated concentrations, be capable of producing an effective antibacterial action without destroying the defense mechanisms of the host cells. Of the various germicides tested, cetylpyridinium chloride diluted in a hypertonic solution to give a concentration of 1:5000 of the quaternary ammonium solution proved to be most valuable as a bronchial lavage in nontuberculous infections. The solution was highly germicidal, had a decided low surface tension action, and was nonirritating in the concentration used.

UROLOGY

Flocks[410] described several solutions of value for the irrigation of kidney pelves containing calcium phosphate stones. He stressed the importance of early diagnosis and treatment of the condition to avoid permanent damage to the urinary system. In addition to 0.8% sulfanilamide and 0.25% acetic acid solutions, Flocks recommended the use of two solutions containing 1:20,000 alkyldimethylbenzylammonium chloride. These contain magnesium oxide, sodium bicarbonate, and either citric acid or malic acid. While some patients tolerated certain solutions better than others, the actual irrigation was considered as important as the dissolving substance. Reference to the use of Flock's solutions in which the quaternary ammonium germicide is used has also been made in a review on urology by Schulte.[411]

Ezickson[412] presented the results of his clinical and bacteriological finding on a series of 35 patients given 130 pelvic lavage treatments with 1:8000 or 1:10,000 dilutions of cetylpyridinium chloride. These cases of urinary tract infections, or urolithiasis, were also studied for urine cultures in 67 instances. Of seven classes of organisms involved in the urinary tract infections, the urine cultures were rendered sterile in six patients following treatment with the quaternary ammonium solutions. No change occurred in eight cases. The ability of cetylpyridinium chloride to prevent infection was evidenced by the fact that the urine culture remained negative after renal lavage. The clinical course of urolithiasis remained unchanged by this form of treatment. The quaternary was used routinely in 260 cases of renal pelvic lavage, ureteral catheterization, and cystoscopy, and for bladder and urethral instillation. There were no signs of infection due to instrumentation and some bladder and urethral infections cleared after use of the germicide. The 1:8000 and 1:10,000 aqueous solution of cetylpyridinium chloride showed no signs of irritation to the tissues.

As an added precaution against postoperative hemorrhage resulting from transurethral prostatic resections in elderly patients, Pool[413] inserted a 24-F catheter with a pear-shaped bag and irrigated the tissue continuously for the first 24 hours with a 1:10,000 solution of alkyldimethylbenzylammonium chloride. Foley[414] advocated daily bladder lavages with antiseptics, such as 1:2000 silver nitrate or 1:10,000 alkyldimethylbenzylammonium chloride solutions, in cases of vesical neck obstruction. In this manner, preoperative complications such as cystitis or ascending pyelonephritis could be avoided. Lewis[415] described the use of the tincture of the same quaternary ammonium compound for the preoperative cleansing of the skin over the perineum prior to repair of recto-urethral fistulas. Marvin[416] also used the tincture of alkyldimethylbenzylammonium chloride to prepare areas for spinal anesthesia in urologic surgery, and Livingstone[417] for the preoperative preparation of the skin in conjunction with the use of penicillin in the treatment of periurethral phlegmon. Reference may also be made to the observation of Walter[346] who used a 10% (1:10) aqueous solution for the preoperative preparation of the scrotum and perineum of three patients with no subsequent evidence of irritation.

In conjunction with sulfonamide regime for the treatment or prevention of chancroid disease, Combes[418] found protection was afforded to the infection by the use of a 10% aqueous solution of alkyldimethylbenzylammonium chloride or a 2% solution in propylene glycol. The quaternary ammonium compound (1:5000) was also found to be a good wet dressing in cases of phimosis and edema.

Harrison[419] cited 13 measures that have been used in the management of nephrolithiasis. He included in the list of procedures irrigations by catheters with buffered citrate solution containing citric acid, magnesium oxide, sodium carbonate, and 1:20,000 aqueous alkyldimethylbenzylammonium chloride. A formula may be substituted for the latter combination which contains malic acid instead of citric acid. These solutions were found to be valuable in dissolving alkaline stones of the bladder and also in certain cases of stones in the renal pelvis.

Alkyldimethylbenzylammonium chloride has been used by Talbot[420] in cystometry and the treatment of vesical dysfunction in paraplegia. The germicide in a concentration of 1:40,000 was found to be mildly antiseptic and nonirritating. In all, 110 cases of spinal cord injury were investigated cystometrically in the study.

Lowsley, Kirwin, and Davalos[421] have recently described a new quaternary ammonium skin antiseptic and disinfectant that is considered of particular value in preparation of the skin for urological surgery. The germicide, benzyl (dodecyl-carbamylmethyl) dimethylammonium chloride, was used with complete success in 530 cases of surgery without evidence of immediate or delayed skin irritation. A 1:500 alcoholic solution of the germicide was used as a skin antiseptic and aqueous dilutions of 1:20,000 to 1:40,000 were employed for bladder irrigations and renal pelvic lavages. While the majority of pathogenic organisms were readily destroyed by the quaternary ammonium germicide, *Ps. aeruginosa* (pyocyaneus) and *B. proteus* were not always eliminated by irrigations with the solution.

OBSTETRICS AND GYNECOLOGY

Mohler and Bishop,[422] in their study of complete abdominal hysterectomies carried out with a standardized technic and the same personnel on 135 patients, used a 1:1000 aqueous solution of alkyldimethylbenzylammonium chloride in the preoperative scrub. Duncan[423] used the same quaternary ammonium germicide in the form of a tincture for swabbing the vaginas of patients both before and after examination under anesthesia in preparation for total hysterectomy. Reference was made also by Duncan to the practice of some operators of leaving a dram or two of alkyldimethylbenzylammonium chloride in the vagina before opening the abdomen in abdominal hysterectomies. Bacteriological studies of the quaternary-treated cases revealed 43 positive and 90 negative smears. There were but slight incidences of minor postoperative complications in the cases studied.

Gates, Williams, and Paxon[424] outlined three factors which they considered will lessen the morbidity percentage in extra-peritoneal section. They list these as: (1) prevention of spill into the abdominal cavity; (2) vaginal instillation; and (3) avoidance of prolonged labor and/or ruptured membranes. In addition to these precautions they advocated the use of antiseptic agents such as 1% acriflavine, 4% 2,7-dibrom-4-hydroxymercurifluorescein, 0.1% sodium ethylmercurithiosalicylate, or 0.1% alkyldimethylbenzylammonium chloride.[s] The routine followed by the authors included instillation of the quaternary ammonium solutions in all cases in labor immediately on admission and repetition of treatment if delivery did not occur in 12 hours. Elective Caesarean cases were also given quaternary ammonium instillations before operation.

Phaneuf[425] presented a study based on 2,328 cases of lacerations of the perineum and their repair. In the operating room the areas were prepared by cleaning the external genitalia with ether and painting them with a tincture (1:1000) of alkyldimethylbenzylammonium chloride. The vagina was also swabbed with the same solution. In the event of considerable vaginal discharge the vulva was first washed with green soap and water and rinsed with a 1:1000 solution of potassium mercuric iodide.

Hill[426] considered the best antiseptics available in 1946 for use during delivery were alkyldimethylbenzylammonium chloride and a proprietary antiseptic known as Dettol. The latter is an antiseptic solution prepared in England which contains the active ingredient, *p*-chloro-*m*-xylenol. The quaternary ammonium solution was recommended for use in the ratio of 1 teaspoon of a 10% concentrate to 1 pint of water (approximately 1:1200). In using the solution for delivery, the external parts were cleansed with swabs soaked in alkyldimethylbenzylammonium chloride. Before repairing a laceration, the surrounding areas were again swabbed with the solution and a swab placed inside the wound to cover it while the patient was draped. A vaginal pack, or rolled perineal pad, wrung out in the solution was placed in the vagina beyond the wound and suturing then carried out in a dry field. Beacham[427] recommended the use of a 1:1000 alkyldimethylbenzylammonium chloride solution for sterilization of the aminotome[t] after washing with soap and water.

Ross[428] used a 1:250 dilution of acriflavine, or 1:1000 solution of alkyldimethylbenzylammonium chloride in the surgical removal of uterine fibroids. On the day before operation, the vagina was prepared as for Caesarean section by cleaning with one of the germicidal solutions on gauze and leaving in a vaginal pack of the same solution overnight. Penicillin in an amount of 300,000 units was given before and after the myomectomy to control or prevent infection, since all submucous fibroids have infected endometria. By using the procedure outlined, Ross performed 900 myomectomies successfully with complete control or prevention of infection. Walter[346] left sponges saturated with a 10% (1:10) solution of alkyldimethylbenzylammonium chloride in the vaginas of five patients for 24 hours following routine dilatation and curettage of the uterus. No irritation to the tissues was noted in these cases.

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[s] See page 198 for trade names and distributors.

[t] Instrument for puncturing the fetal membrane.

While Schmidt[371] early (1935) described his experiences on the use of a quaternary ammonium germicide in trichomonas vaginalis infections, the first detailed clinical reports on the use of alkyldimethylbenzylammonium chloride in conjunction with other chemotherapeutic agents for the treatment of the disease appeared in the publications of Rodecurt.[429-434] In the first article mention was made of the successful use of a preparation containing the active principle acetylaminohydroxyphenylarsonic acid in conjunction with a swabbing regime with the quaternary ammonium germicide for treating leukorrhea. The trichomonads were readily cleared by this combined therapy. In his second publication, Rodecurt[430] reported on the successful use of alkyldimethylbenzylammonium chloride in the treatment of vulvovaginitis infantum which was diagnosed as due to a trichomonas infection in children ranging from two to seven years of age. Due to the inherent difficulties encountered in treating these young patients internally, application of medication to the external genitals resulted in improvement, which was attributed to the fact that the infection is of a vestibular leukorrhea or trichomonas vulvitis.

Subsequent publications by Rodecurt[431,432,433] present additional evidence of the successful use of alkyldimethylbenzylammonium chloride in combination with other forms of chemotherapy for the treatment of trichomonas vaginitis and endometritis, as well as in the treatment of leukorrhea of the cervix. Emphasis was placed on the general application of relatively strong concentrations of the quaternary ammonium germicide to the cervix, vulva, anus, and rectum. One publication by Rodecurt[434] reported a case of trichomoniasis in man transmitted by a woman infected with trichomonas. Genito-urinary manifestations of burning upon urination was one of the clinical symptoms recorded. The condition was apparent several weeks after the initial infection. Upon examination of the urine and exudate, the protozoal cells were noted to have assumed peculiar morphological changes. Unlike the typical trichomonads observed in specimens obtained from females, the cells from male specimens were much smaller and appeared to be in a "resistant" stage. The use of repeated lavages daily with alkyldimethylbenzylammonium chloride solution successfully combated the infection in man.

Greenblatt[435] also included a 1:2000 aqueous solution of alkyldimethylbenzylammonium chloride among various other compounds for urethral irrigations in the treatment of trichomoniasis. He found that the concentrations of quaternary given, as well as 1:1000 acriflavine, were nonirritating and could be used where mild cauterization of the cervix was indicated.

Brown[436] pointed out that in the management of difficult labor the prevention of infection by thorough preparation of the obstetric patient with soap and water scrub is still of utmost importance. This should then be followed by flushing the field with a 1:2000 solution of bichloride of mercury or 1:1000 aqueous alkyldimethylbenzylammonium chloride. He advocated that this technic should be used previous to vaginal examination and delivery.

Shadel[437] also recommended the use of a 1:1000 aqueous solution of alkyldimethylbenzylammonium chloride after the usual soap and water preparation for surgical operations of the perineum in a method he used in obstetric analgesia and anesthesia. Hill and Butler[438] considered the quaternary ammonium solution mentioned to be the most effective of all the antiseptics available for use in the obstetric field. They cautioned, moreover, that the cationic germicide should not be used in the presence of soap.

Brief mention may be made of the use of the quaternary ammonium germicides in pediatrics. Nielson, Ferris, and Logan[439] reported a case of gangrene involving the penis, scrotum, and buttocks of a newborn child. The gangrenous region of the buttocks sloughed on the eighth day leaving two deep ulcerative wounds. Penicillin in doses of 5,000 units were given intramuscularly to the infant every 3 hours for 39 days and sterile dressings wet with *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride were applied to the gangrenous areas continuously for four days. Due to a vesicular skin eruption, which was attributed to the quaternary ammonium solution, this treatment was discontinued. However, upon substitution of the latter germicide by alkyldimethylbenzylammonium chloride the deep ulcerative infected wounds were controlled and healing occurred by granulation.

Hoffert[440] described a new plastic nipple shield that filled the need for a safe, simple standardized method of nipple care. After the daily cleansing of the breast, the inside of the shield is cleansed with 70% alcohol or 1:1000 aqueous alkyldimethylbenzylammonium chloride and the shield then applied. Before each breast feeding the inside of the shields are swabbed with 70% alcohol or the quaternary ammonium solution and placed clean side up on a table. After nursing, the shields are reapplied. By changing to the routine of the plastic nipple shields there was a notable decrease in sore and fissured nipples of the patients.

OPHTHALMOLOGY

Barr[441] reported on the use of a quaternary ammonium solution along with other antiseptics, in the treatment of diseases of the conjunctiva. With particular reference to acute catarrhal conjunctivitis, also called "pink eye," due to pneumococci or Koch-Weeks bacillus, Barr recommended that the conjunctival sac be kept free of purulent material by use of boric acid flushes. These should be followed by alkyldimethylbenzylammonium chloride or some other suitable antiseptic such as an organic mercurial, Protargol or Optochin, which are especially indicated in the pneumococci cases.

Chaimov[442] included a solution of a quaternary ammonium compound among the diluents that could be used in preparing solutions of penicillin. Although no data were given as to the advantages of alkyldimethylbenzylammonium chloride over normal saline or buffer solutions, this may be the first report to suggest that the antibiotic is compatible with a quaternary ammonium compound. Higgins,[443] on the other hand, used penicillin alone in one case of keratitis and one of conjunctivitis, and found both conditions yielded to treatment with the antibiotic agent. In the latter condition, Argyrol, alkyldimethylbenzylammonium chloride, and sulfathiazole ointment had been used previously without success.

Probably the greatest use to which a quaternary ammonium germicide has been put in ophthalmology is to speed the absorption of ophthalmic drugs. O'Brien and Swan[444,445] used a 0.03% of alkyldimethylbenzylammonium chloride solution as the vehicle for doryl in inducing parasympathetic stimulation. Doryl is the name of carbaminoylcholine, a lipoidal, insoluble, surface-inactive compound. The drug is one of a group of synthetic choline derivatives whose action in the eye simulates stimulation of the oculomotor nerve by elaboration at the nerve endings of acetylcholine, which in turn is destroyed by cholesterinase. Since doryl is not destroyed by the latter enzyme, preference is given to its use over the other choline derivatives. Due to its low solubility and poor penetrative properties, O'Brien and Swan used wetting agents, and in particular alkyldimethylbenzylammonium chloride, to enhance absorption of the drug. Reference to the use of the quaternary ammonium compound in conjunction with doryl has also been made by Albough and Brady,[446] Venable,[447] and Wolfe[448] in the treatment of primary or chronic simple glaucoma. An excellent discussion on the use of alkyldimethylbenzylammonium chloride as a wetting agent to enhance penetration of choline drugs into the eye has been presented by Modell.[449,450]

Boyd[451] found the surface, or wetting, action of a 1:5000 aqeuous solution of alkyldimethylbenzylammonium chloride would also increase the speed of penetration of physostigmine in ocular tissues. The salicylate salt of the latter drug was found to be incompatible with the quaternary ammonium solution, and therefore use was made of the sulfate salt of physostigmine in these studies.

The favorable reports by O'Brien and Swan on the use of wetting agents for increasing the penetrating power of ophthalmic drugs prompted Bellows and Guttman[452] to undertake a study on the use of the same agents in conjunction with sulfa drugs in ophthalmology. These investigations involved application of a paste, prepared from sulfathiazole and an aqueous wetting agent, to the cornea and conjunctival sacs of normal rabbits. After the paste had remained in the eye for approximately 1 minute, the cornea and the sac were irrigated to remove the drug. The aqueous humor was then aspirated and analyzed for sulfa content. Several cationic wetting agents and a single quaternary ammonium compound were used in the study. All were found to cause an increase in penetration of sulfathiazole in the ocular fluid and tissues. Since only a 1:1000 aqueous solution of alkyldimethylbenzylammonium chloride was available, the data on this single quaternary were not conclusive. Thus, the other agents tested caused a considerable increase in the penetration of sulfathiazole over that noted when the quaternary ammonium solution was used.

Hind and Goyan[105] studied the role of hydrogen ion concentration and buffer systems used in the preparation of ophthalmic solutions. While previous investigators had recommended the use of esters of para-hydroxybenzoate as preservatives in ophthalmic solutions, Hind and Goyan found that effective mold-inhibiting concentrations of these esters would produce a stinging sensation when applied to the eye. A satisfactory substitute for the esters was a 1:50,000 concentration of alkyldimethylbenzylammonium chloride. This quaternary was used for several years for preventing mold growth in ophthalmic solutions with no apparent indication of irritation to the eye.

Haffly and Jensen[453] suggested the use of an alcohol-acetone tincture (1:1000) of alkyldimethylbenzylammonium chloride for sterilizing the stopper surfaces of vials of ophthalmic solutions. The detergent and germicidal action of the quaternary ammonium solution effectively sterilized the corked surface within a matter of several seconds. An aqueous 1:3000 solution of the germicide was also used effectively by Berens[454] for the sterilization of instruments, sutures, and dressings in cataract surgery. In describing a new model of contact glass for gonioscopy, Troncoso[455] suggested the use of a 1:1000 dilution of *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride as a suitable cleansing agent for the lenses. No mention was made in the article to the germicidal advantages of the use of the quaternary ammonium solution over other wetting agents. Obrig[456] added 1:1000 of *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride to a solution containing 30 ml. of a protein hydrolysate, 70 ml. of 2% methyl cellulose, and 2 grams of potassium carbonate and found this mixture would markedly increase the time of wearing contact lenses before clouding would occur.

Greear[457] described a surgical procedure in which the buccal mucosa is used to restore the orbital socket. In the treatment of the contracted socket the entire face is prepared with soap and water and finally with 1:1000 tincture of alkyldimethylbenzylammonium chloride. While dissecting the socket a packing of gauze wet with 1:5,000 aqueous solution of the quaternary is kept in the mouth throughout the operation.

In the office management of corneal foreign bodies, Hauser[458] described the routine he used in examining and removing the foreign agent. Where antiseptics are indicated if the conjunctiva does not appear clean, he suggested the use of a 1:4000 aqueous solution of alkyldimethylbenzylammonium chloride and 50,000 units per ml. of penicillin drops in normal saline.

OTOLOGY

Several references have appeared in the literature on the application of quaternary ammonium solutions to the external ear and the auditory canal. Lowry[459] found 1:5000 aqueous alkyldimethylbenzylammonium chloride to be an effective solution against fungus infections of the canal. A single daily application of the solution, in addition to being effective in controlling the condition, proved also to be harmless if the eardrum was intact, soothing and painless. The most severe fungus infection required six days of treatment before the ear returned to normal; mild cases required three days. To prevent reinfection, treatment was continued for 10 days. From 50 to 100 ml. of the 1:5000 aqueous solution were used daily. Solutions of 1:1000 concentration or stronger caused desquamation of the tissues. No cotton ear-plug or other medication was necessary in conjunction with the quaternary ammonium treatment.

Senturia and Peugnet[460] described a method of treatment for "aero-otitis externa," a syndrome resulting from the use of ear plugs in flight, in which a quaternary ammonium solution or an organic mercurial antiseptic was used. The procedure consisted of incising the hemorrhagic bullae that occurs in the canal wall, and then inserting a cotton wick saturated with a 1:1000 aqueous alkyldimethylbenzylammonium chloride, or sodium ethylmercurithiosalicylate, into the auditory canal. Complete healing of the subsequently dried canal required about 10 days.

Senturia and Doubly,[461] in a lengthy experimental study simulating therapy of otitis infections in practice, defined and classified the various vehicles, action of vehicles on therapeutic agents, transferability of therapeutic agents from vehicles, etc. The vehicles consisted of a wide range of oily and aqueous agents including alkyldimethylbenzylammonium chloride, *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride, polyethyleneglycol, etc. They noted from their studies that the most effective antibacterial preparation for *S. aureus* was a mixture consisting of penicillin, streptomycin, and one of the quaternary ammonium germicides. The action of the same preparation was less striking against *Ps. aeruginosa.* In tests with combinations of vehicles and the antibiotic agents, the quaternary ammonium compounds were found to be incompatible with gramicidin.

As a substitute for 50% or 70% alcohol in the treatment of otitis externa, Mayberry[462] proposed the use of an organic mercurial antiseptic, hexylresorcinol, or a 1:1000 solution of alkyldimethylbenzylammonium chloride. The solution used should be retained in the ear by means of a saturated wick but after the ear drum has been opened nothing should be used for one or more days. In the event drainage is prolonged, the recommendations were given to irrigate with warm physiological saline followed by drying of the canal and instillation with 50% alcohol or 1:10,000 alkyldimethylbenzylammonium chloride.

Fox[463] also recommended the use of alkyldimethylbenzylammonium chloride (1:10,000) where an antiseptic was required for irrigating the ear. In listing the advantages of the quaternary ammonium solution over other germicides, he included its low surface tension, which would contribute to a cleansing action, along with the nonirritating property to tissue.

ORALOGY

An early reference to the use of a quaternary ammonium germicide for the prevention of transferring of pathogenic bacteria or reinfection of the oral cavity appeared in a publication by Rothenberg.[464] This investigator referred to the use of alkyldimethylbenzylammonium chloride for sterilizing tooth brushes. Since the germicide mentioned was readily soluble in water, nontoxic, stable, and harmless to tooth brushes, with an accompanying killing rate of 2½ minutes against most pathogenic organisms, these attributes favored the use of the compound for tooth brush sterilization.

Miller and his associates[465] found alkyldimethylbenzylammonium chloride to be highly effective in inhibiting the metabolism of organisms found in lesions of dental caries, or in plaques associated with the lesions. In the first of the series of papers by these investigators, the inhibitory action of the quaternary ammonium was studied on the respiration and glycolysis of pure cultures of lactobacilli, *M. tetragenus*, Gram-positive diplococci, and a yeast of the genus Monilia. A pronounced effect against the organisms was noted even when the quaternary was used in dilutions as high as M/10,000 to M/50,000. The inhibition was demonstrated to be complete and irreversible.

Based on the preliminary observations just mentioned, Miller, Muntz, and Bradel[466] studied the effects of alkyldimethylbenzylammonium chloride on the aerobic glycolysis of bacteria in plaque material *in vitro* and *in vivo*. Their investigation revealed that a 1:3000 solution of the quaternary ammonium compound would suppress aerobic glycolysis of homogenized plaque material *in vitro* and also prevent any significant decrease of pH. They were also able to demonstrate that a 1:500 solution of the germicide applied *in situ* to dental plaques of patients would reduce the capacity of the plaque material to produce lactic acid upon subsequent incubation in glucose solution.

Subsequent studies by Muntz and Miller,[467] and Stephan and Miller[468,469] confirmed the early finding on the effects of alkyldimethylbenzylammonium chloride on the glycolysis of homogenized plaque material. However, it was noted that upon using intact plaque substance in their tests the quaternary ammonium compound did not penetrate entirely into these large agglomerates and prevent completely their ability to form lactic acid from glucose. Included in one of the studies[468] to prove these points were *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride, *N*-*N*-*N'*-*N'*-tetramethyl-*N*-*N'*-didodecyl-β-hydroxy-propylenediammonium bromide, and an anionic detergent, 7-[sodium alkylsulfate (alkyl-3,9-diethyltridecanol 6)]. In their last publication on this subject, Stephan and Miller[469] found that a 45% solution or suspension of urea significantly prevented, as well as arrested, dental caries in a controlled group of human subjects. In the same investigations, a 1:1000 aqueous solution of alkyldimethylbenzylammonium chloride was somewhat less effective than urea in controlling dental caries. Yet in this study the quaternary ammonium solution was found to be far superior in controlling dental caries than the commercial dentifrices personally selected by the individuals making up the control group.

Huyck[470] found cetylpyridinium chloride in a 1:4000 concentration to be bacteriostatic and bactericidal for bacteria found in the oral cavity. While its activity was not compared with that of other quaternary ammonium derivatives, he concluded from his observations that this germicide was more effective than those reported by Hanke[471] (National Formulary antiseptic solution, 3% hydrogen peroxide and two organic mercurial germicides).

A survey was made by the Medical Department of the U. S. Army[472] to determine the cause and prevention of dental caries. Among the various agents tested were a liquid dentifrice containing 0.1% alkyldimethylbenzylammonium chloride, gelatin lozenges containing 5000 units of penicillin, and synthetic vitamin K. The results of this investigation were not conclusive inasmuch as examination of the individuals over a one-year period revealed that the increase in caries was as great in the experimental groups as in the controls.

Slanetz[473] compared the relative efficiencies of different sampling methods and medias used to estimate the number of bacteria in the mouth of human subjects. Included in this investigation was the evaluation of several antibacterial agents on the bacterial flora of the oral cavity. These test substances included a glycerite of hydrogen peroxide (0.75, 0.5, and 0.37%), penicillin and a mouth wash solution containing cetylpyridinium chloride. Of the three preparations tested, penicillin in the form of troches produced the most marked reduction in the number of bacteria normally found in the mouth. The remaining two agents resulted in a continual reduction of the oral flora, however, large numbers of bacteria usually persisted when these disinfectants were employed.

DERMATOLOGY

There are but scattered references in the literature on the use of quaternary ammonium germicides in the treatment of fungus infections of the skin. Mention has already been made of the observations of Lowry[457] who considered a 1:5000 aqueous solution to be an effective agent for the treatment of fungus infections of the ear. Thurmon and Brown[474] used an aqueous solution of alkyldimethylbenzylammonium chloride, in cases of pustular dermatophytosis and tinea of the external ear, for sponging the affected areas either before or after application of glycerite of hydrogen peroxide which was under investigation. While the results of treatment with the latter are given in a variety of dermatologic infections, mention was not made of the reason for the application of the quaternary ammonium compound in conjunction with glycerite of hydrogen peroxide in the two specific infections.

Epstein[475] considered alkyldimethylbenzylammonium chloride to be an effective antiseptic for the management of dermatitis in general practice. He recommended the use of a 1:1000 aqueous solution of the germicide or a lotion in which the quaternary ammonium compound was one of the components. Reference is also made by Epstein to the fact that some patients can tolerate concentrations as high as 1:200 of the germicide. Results less favorable than those just mentioned were reported by Hopkins *et al.*[476] These investigators studied the fungistatic action of a number of fungicides in the treatment of dermatophytosis. Much was hoped of the quaternary ammonium compounds because of their effectiveness in bacterial infections and because they appeared to have a fungicidal action when tested *in vitro*. However, in clinical trials of alkyldimethylbenzylammonium chloride and cetylpyridinium chloride only a low percentage of the cases studied by Hopkins and his associates became clinically clear and fungus negative.

In comparing therapeutic methods for treating dermatophytosis and other forms of intertriginous dermatitis of the feet, Weidman and Glass[477] also found that alkyldimethylbenzylammonium chloride was ineffective in treating these infections. The highest concentration used in this study was 1% of the quaternary ammonium compound. Mention may be made that in the controlled study on 117 individuals, Weidman and Glass found metacresylacetate to be the most effective, followed in order of activity by simple boric acid powder.

Bereston and Benteen[478] described a treatment for acne in which exteriorization of the cystic or abscess-like acne was carried out following preparation of the skin with soap and water, ether, alcohol, and an antiseptic such as sodium ethylmercurithiosalicylate or alkyldimethylbenzylammonium chloride. The predominant organisms found in the lesions were aerobic and anaerobic staphylococci and *S. pyogenes*. Barr and Kam[479] obtained some improvement in the treatment of dermatitis repens by the local application of a 1:3000 aqueous solution of alkyldimethylbenzylammonium chloride and intramuscular injections of sodium penicillin.

Morris[480] advocated the use of 1:1000 cetylpyridinium chloride, alkyldimethylbenzylammonium chloride, or *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride in industrial medicine as wet dressings or compresses for eczematized dermatoses. Rake and Dunham[481] used an *in vitro* technic to evaluate the activity of disinfectants, and chemotherapeutic and antibiotic agents on the bacterium of granuloma inguinale. They were able to demonstrate that the marked activity of antimony and potassium tartrate in protecting embryonated chicken eggs experimentally infected with *Diplococcus granulomatis* parallels the clinical findings in respect to the activity of these compounds in the treatment of cases of granuloma inguinale. Low concentrations of mercurous chloride and mixtures of copper sulfate and alkyldimethylbenzylammonium chloride also prevented infections in the eggs. Rake and Dunham inferred that these compounds may be useful when applied locally for prophylaxis against this disease.

GERMICIDAL AEROSOLS

Bryson and Grace[109,110,111] reported on the use of aerosol therapy in the treatment of respiratory disease in which alkyldimethylbenzylammonium chloride was used as the solvent for penicillin and streptomycin which were administered by inhalation. Prior to inhalation of the aerosol, 0.5 ml. of 0.25% Neosynephrine is given in nebulized form and then 100,000 units of penicillin in 3 ml. of a

1:1000 solution of the quaternary ammonium germicide, or the same amount of penicillin plus 200,000 units of streptomycin in the same volume of the germicide. The advantages given for the use of alkyldimethylbenzylammonium chloride as a solvent for the antibiotics include the factors of detergency, emulsifying properties and reduced surface tension provided by the solution. Pus and cellular débris were broken up by the surface-active agent. Bryson and Grace presented tables on the clinical findings of the use of the antibiotic-quaternary ammonium aerosols in a series of 34 patients. A table also shows comparative assays on *S. aureus* of mixtures of penicillin and the quaternary ammonium germicide, and on each agent alone. In listing the various conditions treated, the authors include chronic bronchitis, tracheobronchitis and bronchiectasis, diffuse pulmonary fibrosis, putrid lung abscess, active pulmonary tuberculosis, and fistula. In one case of fistula a streptomycin-quaternary ammonium mixture closed the fistula of eight-year duration and which has since remained healed for one year. In the summary of their report Bryson and Grace consider their observations on the use of antibiotic-quaternary ammonium aerosol therapy as suggestive but promising, particularly in tuberculosis and empyema.

Fulton and his co-workers[112] presented a detailed report of their investigations on the development of liquefied gas—germicidal aerosols in which several quaternary ammonium germicides were combined with dipropylene glycol. Many of the quaternary ammonium compounds were found to have undesirable features to be included in an aerosol formula with the solvent mentioned. In some instances the quaternary ammonium agent proved to be very irritating to the mucous membranes of the upper respiratory tract of human subjects. Other compounds were insoluble or resulted in colored solutions when combined with dipropylene glycol. The quaternary ammonium germicide that was found to be the most desirable for inclusion in an aerosol formula was cetyltrimethylammonium bromide. The resulting aerosol mixture was but mildly irritating, caused no difficulties such as corrosion of metal containers, produced no color, chemical reaction, or precipitation. Fulton demonstrated, moreover, that while aerosols containing dipropylene glycol alone were very effective in reducing the bacterial counts of air they were not germicidal for bacteria on surfaces. The addition of cetyltrimethylammonium bromide to the dipropylene glycol aerosols gave the desired effects on surfaces as well, and showed equal activity against *E. coli* and *S. aureus*.

9. Practical Application—General Disinfection

EATING AND DRINKING ESTABLISHMENTS

While the early application of the quaternary ammonium germicides was in surgical procedures, the evidence that was accumulated on the high germicidal action of the compounds against pathogenic as well as nonpathogenic bacteria, led to their application as general disinfectants in eating and drinking establishments and in food processing plants.

Krog and Marshall[252] were probably the first to report on the use of a quaternary ammonium solution for sanitizing eating and drinking utensils. In a series of swab test studies they found a 1:5000 solution of alkyldimethylbenzylammonium chloride would readily destroy bacteria present on utensils. They proposed the use of a soap and water wash followed by an immediate rinse in a quaternary solution, or preferably immersion of the utensils in a plain water compartment prior to treatment with the germicide. The small amount of detergent or soap carried over, in even the two-compartment wash, had no effect on the potency of the sanitizing solution. Krog and Marshall noted a 1-minute exposure was sufficient for sanitizing utensils. The stability of the quaternary ammonium compound, even at temperatures above 70°F., was given as one of the factors favoring its use for sanitizing purposes.

Walter and Hucker[254] conducted a survey in a locality in which less than half of 34 taverns had hot water available for the rinsing of beverage glasses. Of the number having hot water available, only one maintained a temperature high enough for sanitizing purposes. Twenty-two of the establishments reported the use of some form of chlorine compound in their rinse waters. Since chlorine solutions corroded equipment, made carbonated beverages go flat in a short time, and gave a chlorine flavor and odor to their beverages, many users objected to their use as rinses. Based on this preliminary survey, Walter and Hucker investigated the possible use of alkyldimethylbenzylammonium chloride as a substitute for chlorine for sanitizing beverage glasses. They were able to demonstrate that proper use of the quaternary ammonium germicide resulted in a marked decrease in the number of organisms and complete elimination of coliform organisms from the rims of glasses.

MacPherson[255] reported on the marked reduction, and in many instances complete sterilization, of organisms on used eating utensils following a preliminary wash in soap and water, a rinse in clean water, and finally a 2-minute treatment in a solution of alkyldimethylbenzylammonium chloride. Drinking utensils were first rinsed in cold water only and then in the quaternary ammonium solution. Note was also made by MacPherson of the use of a 1:5000 solution of the germicide in ice-cream scoop containers which, on occasions, may contain millions of bacteria per milliliter in ordinary plain water. With the introduction of the quaternary ammonium solution, the counts could be reduced to less than 10 per ml., even when used over a period of 24 hours. Reference was made, therefore, to the effectiveness of the sterilizing solution even in the presence of the large amount of organic matter found in ice-cream scoop containers.

In a large series of field tests, Mallmann, Kivela, and Turney[256] compared the activity of sodium hypochlorite, chloramine T and several quaternary ammonium germicides in sanitizing beverage glasses. Their data revealed that the quaternaries were as effective or slightly more active than chlorine in reducing the bacterial counts of used glasses. Both types of germicides were definitely superior to slowly acting chloramine T. Furthermore, while the residual chlorine of sodium hypochlorite fell approximately 26% during the study, the residuals of the quaternary ammonium solutions were the same at the end of the runs as they were at the beginning. In addition, it was noted that the presence of certain cationic neutralizing agents in the rinse waters did not materially interfere with the sanitizing action of the quaternary ammonium germicides. On the basis of their finding, Mallmann and his associates considered 150 ppm. of a suitable quaternary ammonium germicide to be as effective as 180 ppm. of available chlorine for sanitizing 800 glasses effectively when exposed to 5-gal. quantities of the solutions for 30 minutes.

Guiteras and Shapiro[482] advocated the use of a quaternary ammonium germicide in combination with a suitable detergent for washing and sanitizing eating utensils in a single procedure. Although their studies involved the experimental use of glass slides that were contaminated with an artificial soil prior to treatment with the bactericidal detergents, certain pertinent data was obtained from their investigations. Evidence was presented to substantiate their theory that when a cationic-active agent (quaternary ammonium compound) is used as a germicide in detergent formulations, it is essential that the detergent be emulsifying instead of saponifying. This is based on the fact that if the alkalinity of the detergent is sufficiently high to saponify the fat, a soap will be formed which will inactivate the germicidal action of the cationic quaternary ammonium compound. A formula was given in which the factors mentioned were taken under consideration.

Davis and Resuggan[483] found that a combination of alkaline detergent salts with a quaternary ammonium compound would fulfill all the requirements of a single-wash system in the washing and sterilizing of glassware. These studies were carried out in the "field" using 20% *N,N*-dioctyl-*N,N*-dimethylammonium bromide with 60% soda ash and 20% hydrated sodium silicate. There is an apparent interest in providing a combination of a detergent-sanitizer for hand and mechanical dishwashing, as well as for use on the dairy farms, which utilizes a quaternary ammonium compound as the sanitizing portion of the formula. Several of these combinations are at present available on the market and some have the desired feature of acting equally well in "hard waters" as in pure (distilled) water[121,484,485] (page 37). A consideration of combinations of alkaline detergents and quaternary ammonium germicides was one of the subjects of general interest at the June 1948 Clinic of the National Sanitation Foundation.[486]

Botwright[487] reviewed the literature on the relative merits of the use of quaternary ammonium compounds as sanitizing agents. The properties inherent in the compounds, i.e., stability, noncorrosiveness, odorlessness, high germicidal action, etc., were given as the advantages of these compounds over chlorine. Reference was specifically made to a quaternary ammonium salt, 9-octadecenyldimethylethylammonium bromide, which Valko and DuBois[34] had described two years prior to the publication by Botwright. The latter writer reported on his studies on the use of the quaternary ammonium compounds for sterilizing drinking glasses. Using chlorine as a control, he noted that with an intermediate rinse the chlorine residual dropped from 100 ppm. to 50 ppm. after 230 glasses were treated. By using the quaternary ammonium germicide as the rinse in the study, the samples from glasses taken at intervals in each series gave logarithmic average bacterial counts of 85 as compared to 2,610 in the chlorine series. Brief mention was also made of a 90 to 100% reduction of bacterial counts following the use of 9-octadecenyldimethylethylammonium chloride on dairy processing equipment. Although not specifically mentioned in the article, Botwright apparently used a concentration of 1:6000 of the quaternary ammonium compound to obtain the results reported in his studies.

Realizing the difficulty restaurant operators encounter in providing heating facilities such that 170°F. water can be delivered to dish-washing machines to sanitize dishes, Mallmann and Zaikowski[488] undertook an investigation on the use of a quaternary ammonium compound as a supplement to heat in the rinse as a sanitizing agent. In laboratory studies they were able to demonstrate that alkyldimethylbenzylammonium chloride in a concentration of 1:10,000 would kill heavy suspensions of *E. coli*, *S. aureus*, and *M. caseolyticus* in a matter of 5 seconds at 70°F. Upon increasing the temperature to 120°F. or higher, the organisms were all killed in 10 seconds by a dilution of 1:15,000. Various neutralizing agents, such as 10% blood serum, equivalent amounts of anionic wetting agents, and soap, were used to eliminate any possible bacteriostatic activity of the quaternary ammonium compound in the studies. In extending these investigations to the practical application of the germicide in the field, Mallmann and Zaikowski introduced the quaternary ammonium germicide in a mechanical dish-washing machine under actual conditions of use in a restaurant. The studies included the sanitizing effects on used dishes of concentrations ranging from 1:10,000 to 1:15,000 of alkyldimethylbenzylammonium chloride when tested at temperatures varying from 120°F. to 140°F. for periods of 10 seconds. They were able to conclude from their findings that the germicide, in a minimum concentration of 1:10,000 in the rinse water, would give the desired results in lowering the bacterial counts of dishes. Evidence was also presented to show that a marked reduction in the bacterial population of the wash water, containing considerable organic matter, will also occur by introducing the quaternary ammonium solution at this point in the dishwashing machine.

In the routine bacteriological examination of eating and drinking utensils Jamieson, Chen, and Willigan[489] encountered considerable loss in the test agar slants due to contamination apparently drawn into their tightly capped containers while cooling. They overcame this difficulty by laying the bottles, while hot from the autoclave, on towels soaked in a 10% solution of alkyldimethylbenzylammonium chloride.

DAIRY INDUSTRY

Probably the first application of the use of quaternary ammonium germicides for the sanitizing of food processing equipment was in the dairy industry. Prior to the introduction of this class of compounds, agents containing chlorine were the only recognized chemical sterilizing solutions used in dairy plants and on dairy farms.

Following their initial studies on the sanitizing effects of alkyldimethylbenzylammonium chloride on eating and drinking utensils, Krog and Marshall[253] extended their investigations on the use of the germicide to include its effects in dairy pasteurizing plants. Bacteriological sampling was made of the milk processing equipment after being cleansed in the usual manner and after treatment with the quaternary ammonium compound. Their studies indicated a concentration of 1:6,000 (170 ppm.) caused a reduction of 60 to 90% of the bacterial counts of the milk-handling and processing systems. They concluded from their findings that alkyldimethylbenzylammonium chloride is definitely bactericidal to the flora associated with dairy processing plants; it is no more corrosive to metal and rubber than ordinary water; it imparts no taste or odor to milk products; and it can be used with safety due to its low toxicity.

Frayer[490,491] compared the efficiency of several commercial nonchlorine compounds in the germicidal treatment of dairy utensils. The study involved an evaluation of the rate of killing action of the compounds on bacteria and the irritating action to the skin. Included among the agents tested was alkyldimethylbenzylammonium chloride. The quaternary ammonium solution in a concentration of 1:2,500 was considered to be satisfactory for use in dairy barns, giving a highly effective germicidal action which was quick acting and apparently nonirritating to the skin. While a sodium *o*-phenylphenol salt was also found to be quick acting in a wide range of concentrations, under practical usage it proved to be "unpleasantly irritating" to the skin of some individuals.

Jamieson and Chen[492,493] described a spray procedure for the supplementary sanitation of milk cans. In their studies the cans were washed manually in alkaline cleansing solutions, steamed and dried, and held approximately 15 hours before testing. Swabs were made of the inside of the cans and covers and the number of bacteria determined by agar-plate counts. The inside of the containers was then sprayed with a chlorine solution, one of two quaternary ammonium germicides, or a nontoxic acid containing a "microbial depressant" (not identified). By using the spray technic, Jamieson and Chen demonstrated that all the sanitizing agents listed would effectively cause an average reduction of 99.87%, or more, of the bacterial counts per swab of the inside treated cans. In general, the swab tests revealed counts of approximately 2.5 millions of bacteria before the spray treatment, and values averaging 143 to 1,060 after treatment. Since the study did not involve a consideration of the superiority of one germicide over another, chlorine in the form of hypochlorite and the two quaternary ammonium germicides, alkyldimethylbenzylammonium chloride and *N*-(acylcolaminoformylmethyl)pyridinium chloride, were used in the single concentrations of 1 ounce to 1 gallon of water in their spray equipment.

Mueller, Bennett, and Fuller[64] tested a large series of surface-active agents with respect to their possible application as germicides for dairy use. Consideration was given not only to their germicidal action but also to their stability under storage conditions, and to their corrosive effects on metals. Raw milk containing large numbers of bacteria was used as the inoculum added to the surface-active solutions. After allowing the latter to act on the milk suspension of bacteria for 5 minutes, a representative sample of the test mixture was plated in agar. Of 42 surface-active agents representing 12 groups of compounds, only the quaternary ammonium and phosphonium salts proved to be sufficiently germicidal and stable to be considered satisfactory for dairy use. Among the surface-active agents found to be only moderately effective in their test were substituted phenols, alkylaryl sulfonates, and aliphatic sulfonates. The compounds showing little, if any, activity were an aryl alkyl polyether alcohol, aliphatic sulfate, aryl alkyl polyether sulfate, several polyoxyalkylenes of fatty acids, and monoesters of polyhydroxy compounds. Although the quaternary ammonium and phosphonium solutions were shown to be noncorrosive, odorless, tasteless, and colorless in dilutions recommended for use, preference was given to the former on the basis of solubility of the salts in water. The phosphonium derivatives did not go into solution readily and often produced cloudy solutions.

DuBois and Dibblee,[47] Johns and Pritchard,[225] and Mull and Fouts[226] studied the effects of quaternary ammonium compounds on the bacterial flora in milk. These investigations were prompted by the fact that, with the wide use of this class of germicides in the dairy industry, some residual amounts of the quaternary ammonium solutions may be left in the equipment, or some unscrupulous persons may attempt the use of the compounds as preservatives or even to reduce the bacterial count of their milk supplies.

In the studies by Johns and Pritchard, tests were made on the preserving action of "critical concentrations" of four quaternary ammonium germicides in milk. The "critical concentration" was defined by these investigators as "that concentration of product which, when added to fresh raw milk and held at 60°F. (15.6°C.) for 18 hours could barely be detected by taste." These concentrations of active ingredient varied in the series of four quaternary ammonium compounds from 7 ppm. to 56 ppm. A formaldehyde control used in the test gave a "critical concentration" of 25 ppm. Their data revealed that one may expect a considerable degree of variability in plate counts in studies of the addition of a quaternary ammonium compound in milk. Thus, an evaluation of the effectiveness of the germicides by this method is almost meaningless. This was further shown by the lack of correlation between the resazurin color number and the plate count, and the methylene-blue reduction time. Johns and Pritchard concluded from their studies that the intentional addition of a quaternary ammonium compound to raw milk by unscrupulous producers exerts only a slight bacteriostatic action in the highest concentration that one may use without detection by taste.

Mull and Fouts also reported on the intentional addition of a quaternary ammonium germicide to milk and the resulting effects on the bacterial flora and taste of the product. Alkyldimethylbenzylammonium chloride was used in a final concentration from 0 to 250 ppm. in various samples of low-count as well as high-count milk. Their studies revealed that the bactericidal action was more noticeable with low-count than with high-count milk and was not affected by pasteurization. In low-quality milk, the quaternary would have to be added to the extent of 200 to 250 ppm. to bring about a significant reduction in the bacterial count. However, when present to the extent of even 10 ppm. in milk, the germicide could be detected by taste as a slight puckery flavor. This increased in intensity to a slight bitterness at 20 ppm. and to a very bitter flavor at 40 ppm. Therefore, according to Mull and Fouts, a producer of low-quality milk would have to add such an excessive amount of the quaternary ammonium germicide to effect an appreciable reduction in high-count milk that the chemical could readily be detected by the average milk consumer.

Mallmann and his co-workers[404] presented a detailed account of field studies, covering a period of one year, in which a number of different methods were used for sanitizing milking machines. In all, 50 producers participated in this investigation. Accurate checks were made over a period of several weeks to determine the average bacterial quality of each milk supply before placing the producers in groups of good, poor, and medium farms. Certain groups in each class were instructed to use a cleaner-detergent and a hypochlorite solution for the routine washing of their milking machines; other groups were to use an alkali in conjunction to the treatment just mentioned; others were instructed to use a quaternary ammonium compound following the preliminary cleaning in a cleaner-detergent. Control groups were to continue the procedure they had previously used in washing their equipment and a final number of producers were to clean their milking machines thoroughly with the cleaner-detergent but were to use nothing more than a clear-water rinse. In comparing the overall picture in bacterial counts for both the summer period and the entire year, it was seen that a marked reduction occurred in the group that received alkyldimethylbenzylammonium chloride as the sanitizing agent. Mallmann's report indicated that the total count for the group using the quaternary ammonium germicide was less than half of that for those using the other sanitizing or rinse procedures. Examination of the data also reveals that, when used as a rinsing solution for milking machines, a 1:6,400 dilution of the quaternary compound was more effective than 200 ppm. (1:5,000) available chlorine in reducing bacterial counts. Mallmann and his co-workers emphasize, however, that the use of a quaternary ammonium compound may be expected only to aid in obtaining good milk from poor producers. If the equipment is improperly cleaned, sanitation cannot be fully attained by any chemical agent due to the interfering action of milk and the lack of penetration of the sanitizing substance.

Hucker[495] prepared a number of mixtures of "modified" nonionic synthetic detergents and quaternary ammonium compounds as cleaner-sanitizers for use in food and dairy plants. Although no mention was made regarding the formulas of these combinations, Hucker found that the "chemically modified" mixtures were effective cleaner-sanitizers for milking machines and could be used in one operation as a cold solution following a cold water prerinse. Simple mixtures of nonionic synthetic detergents and quaternaries which were not properly treated or "modified" were not effective as germicides and, furthermore, were responsible for the deposition of slime on milking machines. Of particular significance to dairy farm or plant operators were the finding that "raw" counts and thermoduric counts could be markedly reduced by the "modified" nonionic synthetic detergents and quaternary ammonium mixtures.

Johns[496] compared the sanitizing efficiencies of various quaternary ammonium compounds and hypochlorites under conditions that may be expected to be encountered in the sanitizing of eating and drinking utensils, food processing, and dairy equipment. He advocated the use of a "glass-slide" technic for determining the efficiencies of germicides. In this procedure organisms are suspended in skim milk that is applied to the glass slide and, upon partially drying, the slide is dipped in the desired concentration of the germicidal solution. At varying time intervals a slide is removed from the test solution, rinsed in sterile water, and plated in nutrient agar. The colonies developing in the medium are then counted or estimated. On the basis of his studies, Johns concluded that it is unsafe to generalize concerning the relative merits of quaternary ammonium compounds and hypochlorites in the sanitizing of food plant equipment. Factors such as types of organisms, pH, temperature, etc., will materially influence the efficiency of the chemical agents. In many respects the hypochlorites appear to respond much more favorably to these factors than do the quaternary ammonium compounds. However, certain inherent advantages in the use of quaternary ammonium germicides, i.e., less corrosion, lack of odor, lower sensitivity to organic matter, less irritation to the skin, etc., favors their use in egg-breaking plants, eating and drinking establishments, and in certain phases of milk processing plants.

The New Jersey Bureau of Animal Industry[497] instituted a plan for mastitis control which involved the use of 400 ppm. of chlorine or preferably 1 ounce of alkyldimethylbenzylammonium chloride to 4 gallons of water for the prevention of *Streptococcus mastitis* infections in cattle. The Bureau and Public Health Departments of many other states have accepted the quaternary ammonium germicide as an alternative for chlorine disinfectants. Evidence is available to show that many of the quaternary salts are as active as chlorine against most bacteria. Therefore, the concentration in parts per million of chlorine recommended for use as a general sterilizing agent is the same as that for the quaternary ammonium compounds.

Mention has already been made (page 29) of recent observations[113] [116] on the use of quaternary ammonium germicides for the treatment of dairy equipment and eating utensils to the effect that certain interfering substances which may be present in some city waters will markedly reduce the bactericidal efficiency of the compounds. Of particular significance is the interfering action of certain hard waters (calcium, magnesium, iron) upon this class of germicides. As an example, data have been presented to show that, while *E. coli* was killed by 20 ppm. in distilled water in 1 minute, 100 ppm. and 250 ppm. of the same germicide were required to destroy the test organisms when suspended in each of two city waters.

Food Processing Plants

Bernstein and Epstein[163] demonstrated that the lauric acid ester of colaminoformylmethylpyridinium chloride can be effectively used as a germicidal detergent in the handling and processing of dill pickles. They cite the advantages on the use of a germicidal-soak in the pickling process over the severe heat treatment that was formerly used to obtain adequate pasteurization or reduction of the heavily contaminated pickles received at the processing plant or salting station. Under controlled field and laboratory tests, Bernstein and Epstein found that samples of unwashed pickles contained 11,500,000 bacteria per ml. of test suspension. After washing with tap water in a presoak, the number was reduced to 60,000 per ml. A final treatment in a 1:6400 concentration of the quaternary ammonium germicide solution reduced the latter count to 3,000 per ml. The authors emphasized the superior detergent action of the fatty acid esters of colaminoformylmethylpyridinium chlorides over that of other quaternary ammonium compounds lacking carboxyl and amide linkages.

Penniston and Hedrick[164,165,166] found that the lauric acid ester of colaminoformylmethylpyridinium chloride was an effective agent for washing and sterilizing dirty poultry eggs. They stressed, moreover, the importance of maintaining the wash water at the same temperature or slightly above the temperature of the eggs to avoid further penetration of the contaminating organisms through the shell. The authors found that the surface of heavily contaminated or dirty egg may contain as many as 250,000,000 viable bacteria. Following a plain water rinse this number may be reduced to 3,000,000 or 6,000,000. Their data revealed that the latter count could be reduced further to only a few thousand or none in a matter of 3 to 5 minutes, by a rinse in 0.05% solution of the quaternary ammonium germicide.

Tressler[498] has pointed out the significance of sanitary precautions in handling sea foods in the preliminary steps to freezing. Along with the maintenance of general cleanliness of the fishing vessels, tables, baskets, bins, etc., he advocated the use of an anionic detergent scrub (which gives suds even in sea water) followed by a thorough rinse with water, and then with hypochlorite or quaternary ammonium germicide solutions. In sterilizing boxes, bins, and other wooden containers, Tressler preferred the use of a quaternary ammonium solution over hypochlorites because the former are more stable and active and also leave a residual amount of antiseptic on the containers.

Brief mention may be made of the possible deodorant action of quaternary ammonium germicides. In a recent editorial,[499] one of the compounds was described as "possessing deodorizing properties which are now finding widespread use in eliminating odors at the source in large manufacturing plants, around garbage dumps, and in polluted streams." One may infer from this statement that the initial properties of the quaternary ammonium compound are to prevent putrefaction by bacteria of the organic matter "at the source."

Mallmann and Churchill[500] conducted a series of investigations on the control of the microbial population in food preparation and storage rooms. These workers demonstrated that if air-borne contamination was the only source of contamination, ultraviolet ray lamps, a carbon dioxide atmosphere, or glycol vapors would effectively suppress the rate of growth of bacteria on surfaces. Since quaternary ammonium germicides exhibited considerable bacteriostatic action on spores, these compounds were preferred for use following good cleansing in preference to hypochlorites. Mallmann stressed, moreover, that the successful use of any form of sanitizer can be done only by treating carefully washed surfaces. Evidence was also presented in the study to show that while high temperatures caused a greater activity of the quaternary ammonium compounds upon spores of *B. subtilis* the differences in activity between 0° and 90°F. were not particularly significant. Mallmann and Churchill concluded from these findings that the quaternary ammonium compounds will give satisfactory results at all temperatures.

Hucker, Brooks, and Van Eseltine[501] compared the activities of a large series of quaternary ammonium compounds against *E. coli*, *A. aerogenes*, *S. aureus*, *Streptococcus cremoris*, *B. subtilis*, a mesophilic flat sour organism isolated from beets, a facultative thermophilic sour culture isolated from peas, and an obligative thermophilic flat sour organism isolated from corn. While there was a wide variation in the relative germicidal efficiency of the various compounds tested, Hucker and his co-workers found that, in general, high concentrations of the quaternary ammonium compounds would even kill resistant spores.

Epstein[502] found that quaternary ammonium solutions were effective in dilutions of 1:2000 to 1:11,000 in destroying several strains of yeast when exposed for 10 minutes at 20°C. A 1:7,000 concentration gave complete sterilization of all strains after a period of 4-hour treatment. Absolute sterilization of unclean beverage bottles was attained with the quaternary ammonium compounds in a concentration of 1:1000 within 10 minutes at 20°C. On the basis of these findings Epstein recommended the use of 1 ounce of a quaternary ammonium germicide to 2 gallons of water as a substitute for chlorine in the sterilization of beverage bottles.

In an editorial appearing in *Wines and Vines*,[503] alkyldimethylbenzylammonium chloride was advocated as a sterilizing agent in place of chlorine for the treatment of winery equipment. A concentration of 1 ounce to 3 gallons of water was recommended for the sterilization of wood tanks, whereas a concentration of 1 ounce to 6 gallons of water was required to treat smooth-surfaced equipment such as tank cars, pasteurizers, filters, etc. For the control of molds and slime forming bacteria on storage and fermenting tanks, application of a dilution of 8 ounces of alkyldimethylbenzylammonium chloride to 1 gallon of water containing a tablespoon of soda ash was recommended every two or three months.

Lehn and Vignolo[504] found that the quaternary ammonium compounds were highly effective in sterilizing brewing equipment. Unlike the "off flavors" produced by other types of germicides that required several hours rinsing with tap water, the quaternary ammonium solutions were simply drained from the equipment or lines prior to use. In a consideration of the use of 9-octadecenyldimethylethylammonium bromide as a sanitizing agent in breweries, Lehn and Vignolo recommended a concentration of 1 ounce of the germicide to 4 gallons of water in the treatment of Lauter tubes, brew kettles, heat strainers, fermenting tanks, and surface coolers. These investigators also advocated the use of the quaternary ammonium compounds in removing mold stains from floors, brew cellars, walls, and ceilings.

DRINKING WATER

Based on early publications on the relative nontoxic action of quaternary ammonium germicides, Kessel and Moore[276] considered the use of these compounds for the emergency sterilization of drinking water. These studies were prompted by the fact that under military conditions the halogens (Cl_2) are often not satisfactory due to their inactivation by organic matter and to their alkalinity. Thus, the high concentration of the hypochlorites required to give adequate safety in rendering water suitable for drinking would be distasteful and toxic. In their preliminary studies the effects of 50 "heteropolar cationic antiseptics" (quaternary ammonium compounds) were studied in the laboratory for their inhibitory action on cysts of *E. histolyticum*. Reference has already been made in an earlier section of this report to the effect that most of the compounds were active in dilutions of at least 1:5,000, some in dilutions of 1:20,000 and 1:80,000. With the data obtained on the latter group of germicides this suggested to them a consideration of the agents for use in sterilizing drinking water, provided some means could be found to inactivate the excess of antiseptic and also to abolish the objectionable taste. The study also led them to propose investigations on these germicides for the decontamination of dishes, fruits, vegetables, clothing, body surfaces, etc.

In a subsequent publication, Moore and Marmorston[505] described a method for the removal of excess quaternary ammonium salts from water after the germicide had effectively sterilized the latter. They were also able to demonstrate in mice that a reversal of germicidal action of the compounds does not occur in the intestinal tract of the animals. To prove the latter point, a heavy suspension of *Salmonella enteritidis* was suspended in sterile water and 1:10,000 and 1:20,000 of alkyldimethylbenzylammonium chloride. These suspensions were fed to mice as their sole source of liquids for a period of three days. Subcultures of the solutions at the time of feeding showed no viable organisms present in the 1:10,000 dilution, whereas a few were evidenced in the 1:20,000 dilution. All the control mice that received the culture in water died with typical enteritis. One of five mice that received the suspension in 1:20,000 germicide died on the eleventh day, whereas all the animals that received the organisms in 1:10,000 of the quaternary ammonium compound survived and showed no untoward effects during the 12-day test period. In the adsorption studies, alkyldimethylbenzylammonium chloride was found to be adsorbed almost completely on colloidal clays. This was evidenced by the lack of taste of the latter and the absence of acute oral toxicity in mice receiving relatively large doses of the adsorbed quaternary ammonium compound. Although Moore did not advocate the immediate use of quaternary ammonium compounds for the sterilization of drinking water, it was intimated that further studies might reveal that these agents could be used for this purpose in human trials. Reference was also made to further studies on the reversal of antiseptic action in the intestinal tract of animals for those compounds intended for use in the food and dairy industry.

QUATERNARY OIL EMULSIONS

During the past several years considerable attention has been directed to physical and chemical means for preventing, or limiting the spread of air-borne infections. For the main part these investigations were instigated by the high incidence of cross-infections of the respiratory tract in contagious wards of hospitals and, in particular, army camps. Van den Ende, Lush, and Edward[506,507] early found the application of a crude oil (spindle oil) to floors would markedly effect a reduction in the number of hemolytic streptococci that would be dispersed in the air during sweeping or dusting of rooms. They demonstrated also that hemolytic streptococci in blankets and bed-clothes would retain their virulence for mice as long as four weeks from the time they were sprayed and dried in the textiles. Attempts were made to incorporate antiseptics with the oil in holding down dust and lint in floor sweepings, however, most of the germicides tested lost their activity when mixed with the oil. The use of a quaternary ammonium germicide, cetylpyridinium bromide, along with a large series of other types of germicides for the treatment of woolens and cotton textiles was found by these investigators to have little, if any, germicidal action on experimentally contaminated fabrics. On the basis of their findings, they hesitated to recommend the use of any germicide for the sanitizing of textile materials. Evidence was presented, however, to show that the use of 30% liquid paraffin for the treatment of streptococcus-contaminated blankets would retain as many as 90% of the organisms even when the material was beaten vigorously.

Palmer and Blow,[508] and Powney and Wood[509,510] used cationic-active agents as a vehicle for the deposition of mineral oil droplets on wool fabrics. The latter investigators found that cetylpyridinium bromide would impart a positive electrical charge on the oil droplets in an oil and water emulsion. Harwood, Powney, and Edwards[511] found that treatment of fabrics in a quaternary oil emulsion would cause a complete discharge of the oil phase in the negatively-charged cotton or wool textile, thereby leaving behind a clear water phase. These "charged" emulsions were more rapidly adsorbed by wool when in an alkaline range of pH (8.0), and reference was also made to the fact that hard water could not be used in preparing the quaternary oil emulsions because of the deposition of calcium and magnesium salts. Although no mention was made in the publication to the germicidal action of the fabrics treated with the quaternary oil emulsion, they, nevertheless, considered that complete exhaustion of the emulsion in fabrics was in itself a process more suitable than the Van den Ende procedure.

Although the English investigators reported on their studies of means of controlling air-borne infections by the use of oil in bedding, and application of oil to floors of hospitals, barracks, etc., as early as 1940, the first reference to similar studies in the United States did not appear in the literature until 1944. These studies were, no doubt, prompted by our entering World War II and the resultant housing of military personnel in close quarters in hospital wards and barracks.

Realizing the importance of controlling diseases of the respiratory tract and the general problem of air-borne infection, a special commission headed by O. H. Robertson was assigned by the U. S. Army to study this subject in the laboratory and in army camps. In one of the first publications by Robertson and his group,[512] the scope of the principles of control of air-borne infections was reviewed. In the same article detailed accounts were given of the effects of glycol vapors, oiled floors, and bedding on the reduction of bacteria in wards and army barracks. For dust-laying of highly polished floors and linoleum, use was made of a solution prepared from 5% urea, 3% Ninol (nonionic wetting agent) and 0.1% alkyldimethylbenzylammonium chloride. This mixture was considered superior to the spindle oil used by the British, for the latter had a tendency to make smooth surfaces slippery and, therefore, a potential hazard to personnel. The urea-Ninol-quaternary mixture dried quickly but held sufficient moisture in the urea crystals, and the Ninol produced wetting of particulate matter, such as dust and lint, which fell on its surface. Reference was also made to the effect that the surfaces treated with the mixture "exerted a bactericidal action." Robertson and his group used a water-oil emulsion for the treatment of bedding. However, no cationic-wetting agent such as quaternary ammonium compound was used in the emulsion. In conjunction with the treatment of bedding and floors for the control of dust and lint, triethylene glycol vapors were used in killing the air-borne organisms of the environments where infections with the microorganisms were taking place. In this and subsequent publications by Robertson and his co-workers,[513,514,515,516] evidence was presented to show that a marked reduction in the incidence of air-borne infections, particularly to hemolytic streptococci, may be obtained when adequate effort is made to reduce the number of bacteria in the air of closed quarters. A general discussion and review of this subject has been made in reports by Loosli[517] and others.[518]

Although Van den Ende and his co-workers failed to show any advantage in the use of cetylpyridinium bromide or other antiseptic agents for the treatment of bedding in hospital wards to reduce the incidence of air-borne infections, Rountree,[519] the U. S. Navy Medical Research Unit 1,[520] and Shechmeister and Greenspan[521] obtained encouraging results on the use of quaternary ammonium germicides for blanket impregnation. In studies on the treatment of hospital blankets with an emulsion containing 70 parts of water, 30 parts of spindle oil, and 1 or 2 parts of cetylpyridinium chloride, Rountree found that the quaternary ammonium germicide in the blanket was responsible for killing dried cultures of hemolytic streptococci. Furthermore, evidence was obtained to show that when blankets were treated with a 1:800 concentration of the germicide the streptococci were killed within a few hours after exposure to the blankets. A reduction of at least 90% of organisms was noted when the blankets were tested for germicidal activity after being stored for 13 weeks. Rountree concluded from her findings that blankets impregnated with an emulsion containing 1:800 cetylpyridinium bromide should destroy hemolytic streptococci, staphylococci, and clostridia in numbers that may be discharged from a patient upon coughing and sneezing.

Shechmeister participated in the extensive study with the U. S. Navy Research Unit[520] on the impregnation of textiles with germicidal emulsifiable oils and later published an article in conjunction with Greenspan[521] on the same subject. Use was made of the principles of Palmer and Blow,[508] and Powney and Wood,[509] in which a quaternary ammonium compound was used to transform a nonionic emulsion (oil) into a cationic emulsion so that in calculated proportions the complete oil phase was adsorbed in the negatively charged fabrics. After trial of several cationic agents, alkyldimethylbenzylammonium chloride was used as the "charging" agent. The studies of Shechmeister revealed also that the germicide not only was necessary for imparting a positive charge to the oil emulsion but also resulted in rendering the blankets germicidal to bacteria coming in contact with them.

10. Miscellaneous

There are scattered references in the quaternary ammonium literature on unusual applications of these compounds. Since the data contained in these articles are not sufficient to warrant an entire section of this review, reference to these publications will be briefly considered in the following paragraphs.

TEXTILE INDUSTRY

Subsequent to the preceding chapter of this monograph, and although not directly concerned with antibacterial properties, it seems appropriate to mention the wide use the quaternary ammonium surface-active agents have received in the textile industry. Of particular significance is their application in this field as "cationic finishing agents" or "cationic-active-softeners" which render fabrics soft and smooth as well as improve the fastness of dyed fabrics to water. A comprehensive review and bibliography on the utilization of the surface-active agents in the textile and related industries has been presented by Ackley.[522]

While a definite germicidal effect can be demonstrated in processed cotton and wool textiles treated with quaternary ammonium germicides, Tiner, Thorp, and Cole[523] have demonstrated that the germicide is precipitated by some substance on the wool of adult sheep and has, therefore, no value as a disinfectant for unwashed wool. Although the nature of the interfering substance was not identified by Tiner, the writer predicts the agent to be a lipid fraction of wool-fat.

Lesser[524] presented a review of the various methods that have been used to prevent mildew on fabrics. Mention is made in the publication of an antimildew product containing as the active ingredient a water-dispersible quaternary ammonium pentachlorophenate. A suggested formula for household use consists of 5 parts of the quaternary ammonium compound, 10 parts of isopropanol, and 25 parts of water. This mixture can be used effectively as a rug shampoo and in dry cleaning establishments. The product can also be employed for the treatment of storage bins, refrigerators and warehouses in which fruits, vegetables and meats are stored.

Borghetty[525] classified the various mildew proofing agents according to their absorbability by fabrics. Alkyldimethylbenzylammonium chloride was classed as a "temporary type" and was considered an effective agent for preserving fabrics during storage or shipment and in the manufacturing process in mills.

LAUNDRY INDUSTRY

It is not to be inferred from the foregoing section on the impregnation of fabrics with an oil-quaternary ammonium emulsion that this is the only means of imparting a germicidal action to the woolen or cotton textiles by the use of quaternary ammonium germicides. Evidence is available in our laboratories to show that the mere rinsing of fabrics in appropriate aqueous solutions of the cationic compounds is sufficient to provide them with a definite germicidal action. The positively-charged cationic germicides combine with the negatively-charged fabrics in such a manner that they become "fixed" on the latter, at the same time permitting a sufficient amount of the compounds to diffuse in nutrient broth or agar medium to inhibit the growth of *S. aureus, E. coli, S. pyogenes,* and fungi (*A. niger*). Washing quaternary ammonium-treated fabrics in cold, warm, or boiling water fails to remove the antibacterial properties of the textiles. Treatment with an anionic solution (soap, etc.) will, however, destroy the bactericidal properties of the cationic agents contained in the cloths.

Mention has already been made of the studies of Barail[367] on the possible irritating action of quaternary-impregnated infants' clothing. Although direct evidence is not available on the adverse effects of such treated fabrics under actual conditions of use, the principle of employing quaternary ammonium compounds in the routine laundry rinse for "sanitizing" towels, bed-linens, wool blankets, etc., appeared to merit consideration of investigation.

Benson and his co-workers[524] described a new treatment for diaper rash which involves the use of a quaternary ammonium compound, *p*-diisobutylcresoxyethoxyethyldimethylbenzylammonium chloride monohydrate, in a final rinse in the laundering of diapers. Diaper rash is an ammonia dermatitis resulting from the bacterial decomposition of urinary urea to give free ammonia. The saprophytic Gram-positive organism which is usually responsible for this reaction is *Bacillus ammoniagenes* which originates in the feces of infants.

Benson found that the quaternary ammonium germicide would inhibit the growth of the latter organisms in agar-cup plates in concentrations as high as 1:110,000. The compound was also found to have a definite antibacterial action on the bacteria in the presence of broth in dilutions up to and including 1:200,000. Diapers treated with a 1:10,000 solution of the quaternary ammonium germicide and inoculated with *B. ammoniagenes* and urine were found to have but a faint odor of ammonia after 20 hours. The untreated diapers, on the other hand, had a strong evidence of ammonia within a storage period of 15 and 20 hours. Clinical studies on the quaternary revealed that concentrations of 1:5,000 could readily be tolerated by infants and newborns when the solution was applied to the skin in the form of wet-patch tests for 24 hours. The recommended concentration for general use in the rinsing of diapers is 1:25,000 of the solution to 2 quarts of water. This volume will effectively treat six diapers. Benson and his co-workers found that by the impregnation of diapers with the quaternary ammonium germicide 49 infants with ammonia dermatitis were cleared of this condition within one week.

The New York City Health Department introduced an amendment to their sanitary code on January 13, 1948 (section 336),[527] involving regulations of public laundries. The latter included those established and operated for service to the general public such as steam laundries, wet-wash laundries, hand laundries, diaper laundries, and rental self-service laundries. Of particular interest to this review are the regulations in the amendment involving the requirements of a "germicidal treatment" during the final rinse period of fabrics whose character or nature cannot withstand the "high temperature process" of laundering (140°F. or greater). In a memorandum issued by the New York City Bureau of Sanitary Engineering (March 1, 1948) the standards and approved methods for the "germicidal treatment" of fabrics are described in detail. The statement is made to the effect that the products used shall not be deleterious to or have an adverse effect on the fabrics that are to be laundered at low temperatures (below 140°F.). It was pointed out that the hypochlorites and chlorine-bearing compounds were, therefore, not acceptable for the "germicidal treatment" or process of laundering.

The approved method, as prescribed in Regulation 8 of Section 336 of the sanitary code, considered all quaternary ammonium compounds of recognized germicidal value as acceptable provided they produced a complete kill of *E. typhosa* or *S. aureus* within 10 min. in a dilution of 1:5,000 at 20°C. The germicides used should be employed in an initial concentration of 150-200 ppm. (1:6,500) and should be added at the beginning of the final rinse period.

An alternative approved method of "germicidal treatment" of fabrics is permitted by the New York City Health Department which utilizes a germicide-detergent combination consisting of a nonionic detergent that is compatible with the quaternary ammonium germicide. Since these mixtures combine the principles of detergency or cleansing properties and germicidal action, the addition of more quaternary during the final rinse is not required.

Lawrence and Murphy[528] used alkyldimethylbenzylammonium chloride in the "germicidal treatment" of soiled clothes in a "rental self-service laundry." The automatic washing machines used were of Westinghouse manufacture and would contain up to 9 pounds of dry laundry. Ten gallons of water is used in the "wash" phase and 7 gallons in the "deep-rinse" phase.

The soiled clothes used were the routine accumulations from several households. Up to the point when the quaternary ammonium germicide was added, 10-ml. samples were withdrawn with a sterile 10-ml. pipet and placed in sterile, dry tubes. All samples taken from the point of the addition of the quaternary ammonium germicide were placed into tubes containing 1.0 ml. of a 1.0% solution of Suramin Sodium, U.S.P. XIII, a quaternary inactivator. The latter was prepared by dissolving the compound in a phosphate buffer solution, pH 7.2. One milliliter of this solution was placed into tubes and autoclaved at 15 pounds pressure for 20 minutes.

The samples were withdrawn from the washer with sterile pipets and brought to the laboratory for plating within 1 hour. One-ml. samples was added to Letheen agar (±40°C.) and plated. The plates were incubated at 37°C., and read at 48 hours. In the cases of the samples that were diluted to facilitate counting of the colonies, and then plated, sterile distilled water was used as the diluent.

It may be well to point out that, in this series of studies, routine washing in the Westinghouse Laundromat involves the use of a single deep-rinse following emptying and extraction of the soap wash. Upon filling the washer with 7 gallons of water, which constitutes the deep-rinse, the water can be observed to have a considerable amount of residual soap absorbed in the clothes, as evidenced by a definite foam on the surface of the rinse water. At our request, in some instances the Laundromat operator included a second deep-rinse in order to reduce the residual amount of soap before the addition of the germicide. The results of this investigation are presented in Table 21.

An analysis of that data given in the table will reveal that the second deep-rinse contains almost as many bacteria as that observed in the first deep-rinse. Furthermore, using an appropriate amount of alkyldimethylbenzylammonium chloride (1:5000) in the first or second deep-rinse, comparable germicidal activity may be obtained in both runs. It is apparent, therefore, that the residual amount of soap that is left in the clothing even after the first rinse will not materially interfere with the action of the quaternary ammonium germicide.

The State of Illinois has a legal ordinance in its sanitary code which requires the sterilization of suits worn by swimmers. Early enforcement of this law was attended with some difficulty because of the lack of information concerning suitable disinfectants that could be used for the treatment of bathing suits. This prompted

TABLE 21

USE OF ALKYLDIMETHYLBENZYLAMMONIUM CHLORIDE IN THE GERMICIDAL TREATMENT OF LAUNDRY

TYPE MACHINE	DETERGENT USED	TYPE OF WASH	WATER TEMP. °C.	SOURCE (POSITION IN RUN) OF SAMPLE	PLATE COUNT (COLONIES/0.1 ML. RINSE)* STRAIGHT	PLATE COUNT (COLONIES/0.1 ML. RINSE)* 1:1000
Westinghouse (7 gal. rinse) (2 deep-rinses)	"70% Soap" (as supplied by Launderette)	Diapers, socks, towels, pillow-cases	40	1st soak	Innum.†	25,600,000
				After soap and wash	Innum.†	200,000
				1st deep-rinse, no quaternary	Innum.†	....
				2nd deep-rinse, no quaternary	Innum.†	....
				2nd deep-rinse, after quat. 1:5000	Sterile	Sterile
Westinghouse (7 gal. rinse) (2 deep-rinses)	"70% Soap"	Diapers, socks, shirts, towels, sheets	40	1st soak	Innum.	5,600,000
				After soap and wash	Innum.	170,000
				1st deep-rinse, no quaternary	Innum.	....
				2nd deep-rinse, no quaternary	Innum.	....
				2nd deep-rinse, quaternary 1:5000	3 col.	Sterile
Westinghouse (7 gal. rinse) (1 deep-rinse)	"70% Soap"	Towels, sheets, pillow-cases	40	1st soak	Innum.	3,600,000
				After soap and wash	Innum.	70,000
				1st deep-rinse, no quaternary	Innum.	....
				1st deep-rinse, quaternary 1:5000	1 col.	Sterile
Westinghouse (7 gal. rinse) (1 deep-rinse)	"70% Soap"	Slacks, dress, pajamas, underwear	40	1st soak	Innum.	22,400,000
				After soap and wash	Innum.	60,000
				1st deep-rinse, no quaternary	Innum.	....
				1st deep-rinse, quaternary 1:5000	67 col.	Sterile
Westinghouse (7 gal. rinse) (1 deep-rinse	Quaternary-detergent combination‡	Shirts, T-shirts	40	1st soak	Innum.	1,500,000
				After wash in quat.-det.	Innum.	30,000
				Middle of deep-rinse	27 col.	....
				End of deep-rinse	10 col.	Sterile
Westinghouse (7 gal. rinse) (1 deep-rinse	Quaternary-detergent combination	Sheets, slacks, towels, socks	40	1st soak	Innum.	80,000
				After wash in quat.-det.	28 col.	Sterile
				Middle of deep-rinse	73 col.	Sterile
				End of deep-rinse	86 col.	Sterile

Westinghouse (7 gal. rinse) (2 deep-rinses)	“70% Soap”	Sheets, pillow-cases	40	1st soak	Innum.	30,000
				After soap and wash	0	Sterile
				1st deep-rinse, no quaternary	160 col.	......
				2nd deep-rinse, no quaternary	Innum.	....
				2nd deep-rinse, quat. 1:10,000	Sterile	Sterile
Westinghouse (7 gal. rinse) (2 deep-rinses)	“70% Soap”	Socks, towels, underwear	40	1st soak	Innum.	200,000
				After soap and wash	Innum.	70,000
				1st deep-rinse, no quaternary	Innum.	....
				2nd deep-rinse, no quaternary	Innum.	....
				2nd deep-rinse, quat. 1:10,000	9 col.	Sterile

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* Counts based on plating of only 0.1 ml. of test samples.

† Innum. Too many to count in plate.

‡ 2 oz. of quaternary (10%)-detergent added to 10 gal. water. Final concentration of quaternary, 1:13,000.

Wallace[529] to undertake an investigation to evaluate substances that might be used to effectively disinfect the suits and which, correspondingly, would have no adverse effect on the fabrics themselves. A tentative study was made in which the germicidal activity of a group of phenolic compounds were compared to the action of alkyldimethylbenzylammonium chloride. The method of testing the compounds consisted of impregnating small square samples of suit material with suspensions of *E. coli, S. aureus*, or *Tr. interdigitale.* The squares were dried in a sterile container for 18 to 24 hours and then placed in a disinfectant dilution. At time intervals of 1, 4, 6, and 10 minutes a square was removed from the test solution, washed in sterile distilled water to rinse off the excess disinfectant, and then cultured in a nutrient medium. Failure of growth to develop in the medium was considered evidence that the organisms had been killed by the disinfecting solution.

Of the series of compounds tested, Wallace found that a 1:200 dilution of benzylphenol, 1:50 sodium-*o*-phenylphenate, and a 1:1000 solution of alkyldimethylbenzylammonium chloride would kill all three test organisms in the times given. Of the latter three disinfectants and in the dilutions mentioned only the quaternary ammonium germicide showed no noticeable effect on a variety of swimming suit fabrics. On the basis of his finding, Wallace considered alkyldimethylbenzylammonium chloride to be the compound of choice for the disinfection of swimming suits. The factors of low cost, water solubility, germicidal activity, colorless and odorless properties, and harmlessness to fabrics were given as the advantages for the use of the quaternary ammonium compounds over that of the phenol derivatives studied.

Brief mention may be made at this point of a program that has been instituted by the Health and Safety Department of the West Allis Works of the Allis-Chalmers Manufacturing Company for the sterilization of protective equipment.[530] The efficiency and applicability of alkyldimethylbenzylammonium chloride sterilizing baths has been proved by more than two years of practical application in their plant. The germicide replaced the less efficient and less desirable formaldehyde fume method that had previously been used for the sterilization of protective equipment. The quaternary solution remained stable for a period of at least one week and was found to have sterilizing properties sufficient to treat respirators, cup-type goggles, spectacle-type goggles, rubber suits, boots, sand-blast helmets, gloves, aprons, etc.

PAPER INDUSTRY

Appling and McCoy[531] studied the relative toxic (antibacterial, antifungal) properties of several classes of disinfectants recommended for use in controlling bacterial slime and molds in the paper industry. They concluded from their findings that the quaternary ammonium germicides *N*-(acylcolaminoformylmethyl)pyridinium chloride and alkyldimethylbenzylammonium chloride were not as effective against the mold *A. niger* as they were against the bacterium *A. aerogenes*. With the organic mercurials and chlorinated phenols, the positions were reversed, with *A. aerogenes* being the more resistant. *B. mycoides,* the spore-forming bacterium commonly associated with paper-mill slime, was the easiest of the three organisms to control by all the groups of disinfectants studied. A comparison of the inhibiting concentrations for the three test organisms and the cost data for the various disinfectants was presented by the authors as a guide to manufacturers for selecting the most desirable compound for controlling contaminations in the paper industry.

MINING INDUSTRY

Note may be made of the application of quaternary ammonium surface-active agents in the mining industry. Among the several publications on this subject are the informative articles by Taggart and his associates.[532,533,534] These investigators considered that the principle involved in the impregnation of fabrics with these compounds from faintly alkaline solutions was the same mechanism of action responsible for their ability to impart a hydrophilic character on ores. Many modern mining industries employ large quantities of quaternary ammonium compounds in the "froth flotation" process of separating minerals. Dean and Ambrose[535] have also presented an excellent review of the mechanisms of action of surface-active agents in ore flotation or "collecting" procedures.

Dryden and Meakins[536] used the principles and theories of ore flotation in their investigations on the treatment of glass and ceramic with quaternary ammonium compounds for the improvement of electrical insulation resistance. An excellent explanation for the so-called "film" that appears on eating and drinking utensils that have been sanitized with a quaternary ammonium germicide is presented in the Dryden and Meakins publication. They pointed out that the adsorption of an unimolecular film of an "amphipathic" (polar-nonpolar) compound on a solid surface will result in the formation of a paraffin-like character that will render the object less readily wetted by water. In this respect, the surface of glass in contact with an aqueous solution is negatively charged and will attract cations present in the medium. Therefore, if "amphipathic" cations (as quaternaries) are present in the solution, the latter become oriented in a manner so that the polar groups are turned toward the glass surface. On the other hand, the hydrogen and metal ions on the surface are continuously exchanged with the cations in the solution. Thus, the diffusion of "amphipathic" cations from the surface back into the solution is blocked as the result of the "cohesion of the nonpolar groups." Dryden and Meakins found that treatment of glass microscopic slides and steatite terminal bushings in chromic acid and then in an aqueous solution of trimethylammonium bromide would result in an improvement in the electrical resistance of these objects when exposed to moist conditions. Their studies were prompted by the failure of much electrical equipment under service in the tropics due to the absorption and condensation of water on the surfaces of insulators.

PRESERVATION OF PLANTS AND ORGANIC MATTER

Weissflog[537] presented a unique method for keeping cut flowers fresh longer by the use of organic nitrogen compounds with a nutrient substance as cane sugar, levulose, glucose, etc. Among the nitrogen substances mentioned were compounds with at least one aliphatic hydrocarbon radical and 10 to 18 carbon atoms attached to the nitrogen. Included in the list were the aliphatic amines, alkyl-, aryl-, or aralkyl-substituted ammonium bases that could be used as such or in the form of salts of inorganic or organic acids. The quaternary ammonium germicides fall in this category as substances that will prevent early putrefaction and spoilage of cut flowers. The advantages given for the use of the aliphatic amines are that, unlike the silver and copper salts, they give no precipitate and no color, appear compatible, and may be of nutritive value to the plants.

Based on the early observations of Kuhn and Jerchel[303] that certain tetrazolium salts would stain viable or living bacteria and plants, Waugh[538] proposed the use of triphenyltetrazolium chloride for determining the viability of trees, shrubs, and cuttings. Treatment of green plants with this compound will result in a change from the green chlorophyll color of the leaves to a distinct red color.

Tice and Moore[539,540] found that low concentrations of quaternary ammonium compounds were effective in preserving gelatin and sucrose solutions, particularly at a pH range that precluded bacterial growth, but not yeasts and molds. Solutions containing both gelatin and sucrose required much greater concentrations of the germicides to preserve the mixtures effectively. They noted, moreover, that considerable differences may be expected in the efficiencies of *p*-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride and cetylpyridinium chloride.

Sanders[541] found that the addition of 1% alkyldimethylbenzylammonium chloride to the usual phenol-formalin solution would effectively preserve embalmed museum specimens that might be attacked superficially by highly resistant organisms. The low cost of the quaternary ammonium germicide as compared to sodium azide was also a factor listed by Sanders which favored the use of the quaternary for the purpose given.

Alkyldimethylbenzylammonium chloride has also been used by Jump and Hutchinson[542] to prevent damage of photographic negatives by microorganisms. Included among the desirable features of the use of the germicide were the nontoxicity to personnel, simplicity in application, and lack of undesirable physical or chemical changes in the negatives treated with the quaternary ammonium solution. Processed negatives dipped in a 1% solution of the germicide were found to withstand six months' leaching from condensation, high humidities and heavy contamination by organisms. Control negatives in the same study showed fungus growth in two days and were completely worthless within a few weeks.

Mention has been made previously in these pages to the studies of Nagel[215] on various aspects of the germicidal action of alkyldimethylbenzylammonium chloride. Although not indicated before in this text, Nagel's observations involved a comparison of solutions prepared from an aqueous concentrate of the quaternary ammonium germicide to solutions prepared from paper sheets impregnated with the compound. His data revealed that solutions prepared from the latter were equally effective in concentrations and bactericidal activity as the samples prepared from an aqueous concentrate. The principle of impregnation of foils or sheets with a quaternary ammonium germicide is the basis of a U. S. patent issued to Wolfert and Daab.[543] This patent proposes the addition of an inactive substance to the active germicide without impairing the disinfecting action of the compound or physical properties of the foils or sheets. It cites, as an example, a combination of equal parts of carbamic-acid-ethylester with dodecylamide of dimethylbenzylammonium chloride acetic acid. These are melted together and the melt applied to sheet-like carriers (paper-ribbon, etc.) by pouring or dipping.

SOAPLIKE PREPARATIONS

Considerable attention has been directed during the past few years to the development of soapless detergents and foaming agents. While there are many products commercially available as detergent substitutes for soaps, most of these preparations are anionic or nonionic in chemical structure and have little, if any, germicidal activity. Kirby and Lontz,[544] however, described a number of disinfectant detergent mixtures which embody both of the features of a cleansing agent along with a definite germicidal activity. These mixtures contain betaines as the detergent factor and a water-soluble bactericidal basic nitrogenous compound. An example of one of these mixtures is *N*-dodecylbetaine with polyhexamethylenedimethylammonium bromide. Evidence was presented to show that a 1:200 aqueous solution of the betaine was devoid of any germicidal activity. Furthermore, while the quaternary ammonium compound alone would destroy *S. aureus* in 10 minutes in a 1:10,000 concentration, the addition of 1% betaine to the quaternary ammonium salt increased its activity to the extent that a dilution of 1:30,000 or greater would destroy the organisms in 10 minutes.

Soaplike preparations in which quaternary ammonium compounds are the germicidal components of the products have also been described by Rawlins.[545] The lathering fractions of the preparations were salts of hexadecylamine and octadecylamine. These mixtures were described as being excellent lathering agents which were nonirritating and, therefore, suitable for toilet and similar use with the added feature of having the germicidal activity of the quaternary ammonium compounds.

POULTRY INDUSTRY

Bolin, Goldsby, and Eveleth[546] used a quaternary ammonium germicide, diisobutylphenoxyethoxyethyldimethylbenzylammonium chloride for controlling protozoan diseases of poultry. The organisms usually associated with the infections of the gastrointestinal tract of birds are *Trichomonas gallinarum* and *Histomonas meleagridis*. These protozoa are responsible for the condition known as "blackhead," or enterohepatitis, which describes a disease syndrome in which the principle lesions are located in the liver and ceca. The quaternary ammonium compound was added to the drinking water or in some instances was administered directly to the birds as a dry powder in capsules. The doses given in either form were found to be relatively nontoxic to birds.

Bolin and his co-workers found that the addition of 1:1,666 to 1:10,000 of the quaternary ammonium compound in the drinking water of turkeys would markedly reduce the incidence of blackhead infections of the poults. A noticeable decline in a mixed infection of histomoniasis and hexamitiasis was also observed in a flock of 2,350 poults that received a 1:10,000 solution of the quaternary in drinking water. The compound was also used successfully in the treatment of a trichomonas infection of the crop, liver, and intestines of pigeons.

The United States Food and Drug Administration[547] were at first reluctant to permit the use of quaternary ammonium germicides in the drinking water of poultry and regarded the compounds as "new drugs." Later it was announced, however, that a direction to keep clean water before the birds was a minimum labeling requirement to insure disinfecting activity.

With the data filed by the manufacturers, and additional technical findings of the veterinarians of the Food and Drug Administration, dilutions of 1:5,000 to 1:10,000 of the quaternary ammonium compounds were considered safe for both chickens and turkeys. The Food and Drug Administration authorities[548] stipulated, however, that the germicides were to be labeled and sold only for disinfecting poultry drinking water. In the event any representation was made that the quaternaries were effective in the treatment or control of a specific disease, the drinking water product would under such labeling be considered a new drug.

Moskey[549] reviewed the decision of the Food and Drug Administration which permitted the use of quaternary ammonium compounds in the drinking water of poultry. He stated, moreover, that the available evidence showed that the quaternary ammonium compounds had no value in prevention of coccidiosis or blackhead in turkeys.

Mention has already been made in the virus section of this monograph to the studies of Tilley and Anderson[296] of the U. S. Department of Agriculture who found quaternary ammonium germicides were highly effective in destroying the virus of Newcastle disease (avian pneumoencephalitis). Although these investigations were carried out in chick embryos one may infer from their finding that the addition of quaternary ammonium compounds to drinking water would also prevent cross-infection of Newcastle disease in poultry.

FISH HATCHERIES

Rackley[550] used alkyldimethylbenzylammonium chloride in the successful treatment of gill disease in trout. A concentration of 1:50,000 of the germicide was added to the pool and the fish allowed to remain in the bath for 1 hour. Little, if any mortality was noted when the germicide was used in the concentration given. A detailed description of the procedure for treating gill disease in large trout hatcheries may be found in the publication by Rackley.

Ordal and Rucker[228] also found that certain quaternary ammonium compounds were effective in controlling gill disease in salmonoid fish. These investigators found, however, that there is a relatively low margin of safety between the effective concentrations that will destroy the myxobacterium, *Chondrococcus columnaris,* and the concentrations which are toxic to fish. In studying a homologous series of alkyldimethylbenzylammonium chlorides, the dodecyl (C_{12}) and tetradecyl (C_{14}) derivatives were found to be relatively more effective when the destructive action against bacteria was compared with the toxicity to fish.

11. Surface-Active Agents and Germicides

QUATERNARY AMMONIUM GERMICIDES

Trade Name	*Chemical Name*	*Distributor or Manufacturer*
ZEPHIRAN	Alkyldimethylbenzylammonium chloride	Winthrop-Stearns, Inc.
ALZAN	Alkyldimethylbenzylammonium chloride	Winthrop-Stearns, Inc.
ROCCAL	Alkyldimethylbenzylammonium chloride (industrial)	Winthrop-Stearns, Inc.
B. T. C.	Alkyldimethylbenzylammonium chloride	Onyx Oil and Chemical Co.
RO-E	Alkyldimethylbenzylammonium chloride (technical)	Onyx Oil and Chemical Co.
RODALON	Alkyldimethylbenzylammonium chloride	Rhodes Chemical Co.
STER-BAC	Alkyldimethylbenzylammonium chloride	Klenzade Research
STERISAN	Alkyldimethylbenzylammonium chloride	Hiland Chemical Products Co.
BIONAL A	Alkyldimethylbenzylammonium chloride	The General Dyestuff Corp.

Trade Name	*Chemical Name*	*Distributor or Manufacturer*
D. P. T.	Alkyldimethylbenzylammonium chloride	Adept Laboratories
ZYLIUM	Alkyldimethylbenzylammonium chloride	Turco Products (Borde Co.)
NO-ROMA	Alkyldimethylbenzylammonium chloride	Baird and McGuire
WILLAT COLD DISINFECTANT	Alkyldimethylbenzylammonium chloride	———
CHEMCIDE	Alkyldimethylbenzylammonium chloride	———
PURINSE	Alkyldimethylbenzylammonium chloride	———
THORAL	Alkyldimethylbenzylammonium chloride	———
AMICIDE	Alkyldimethylbenzylammonium chloride	———
KECIDE	Alkyldimethylbenzylammonium chloride	———
Q. A. C.	Alkyldimethylbenzylammonium chloride	———
WECOCIDE	Alkyldimethylbenzylammonium chloride	———
NOPCO-QCL	Alkyldimethylbenzylammonium chloride	National Oil Products
HYAMINE 1622	Diisobutyl (or *p*-tertiaryoctyl)-phenoxyethoxyethyldimethylbenzylammonium chloride	Rohm and Haas Co.
POLYMINE D	Diisobutyl (or *p*-tertiaryoctyl)-phenoxyethoxyethyldimethylbenzylammonium chloride	Rohm and Haas Co.
DISILYN	Diisobutyl (or *p*-tertiaryoctyl)-phenoxyethoxyethyldimethylbenzylammonium chloride	Rohm and Haas Co.
PHEMEROL	Diisobutyl (or *p*-tertiaryoctyl)-phenoxyethoxyethyldimethylbenzylammonium chloride	Parke-Davis and Co.
TIMSEN	Diisobutyl (or *p*-tertiaryoctyl)-phenoxyethoxyethyldimethylbenzylammonium chloride	Theo. Ross Associates
GERMEX	Diisobutyl (or *p*-tertiaryoctyl)-phenoxyethoxyethyldimethylbenzylammonium chloride	Cutler Chemical Co.
MERCO	Diisobutyl (or *p*-tertiaryoctyl)-phenoxyethoxyethyldimethylbenzylammonium chloride	Merriam and Co.
EMULSEPT	*N*-(acylcolaminoformylmethyl)-pyridinium chloride	Emulsol Corp.
STERILEX	*N*-(acylcolaminoformylmethyl)-pyridinium chloride	Pittsburgh Chemical Laboratories
TREET BACTERICIDE	*N*-(acylcolaminoformylmethyl)-pyridinium chloride	———
HQ DISINFECTANT	*N*-(acylcolaminoformylmethyl)-pyridinium chloride	———

Trade Name	*Chemical Name*	*Distributor or Manufacturer*
ML-10	*N*-(acylcolaminoformylmethyl)-pyridinium chloride	—
COCCOCIDE	*N*-(acylcolaminoformylmethyl)-pyridinium chloride	—
DREWICIDE	*N*-(acylcolaminoformylmethyl)-pyridinium chloride	—
MIRACLE 19	*N*-(acylcolaminoformylmethyl)-pyridinium chloride	—
LANCO 706	*N*-(acylcolaminoformylmethyl)-pyridinium chloride	—
EMCOL 888	Alkylarylpyridinium chloride	Emulsol Corp.
EMULSOL 660B	Laurylpyridinium chloride	Emulsol Corp.
EMULSOL E607M	*N*-(myristic acid ester of colaminoformylmethyl) pyridinium chloride	Emulsol Corp.
ONYXIDE	9-octadecenyldimethylethylammonium bromide	Onyx Oil and Chemical Co.
QUARTOL	9-octadecenyldimethylethylammonium bromide	Onyx Oil and Chemical Co.
AMERSE	9-octadecenyldimethylethylammonium bromide	Vestal Chemical Co.
THORAL	9-octadecenyldimethylethylammonium bromide	Turco Products (Borden Co.)
RO-A	9-octadecenyldimethylethylammonium bromide	Onyx Oil and Chemical Co.
RO-B	9-octadecenylethyldimethylammonium bromide (technical)	Onyx Oil and Chemical Co.
RO-C	Cetyldimethylbenzylammonium chloride	Onyx Oil and Chemical Co.
RO-D	Dodecyldimethylamine oxide	Onyx Oil and Chemical Co.
RO-F	9-octadecenyldimethylamine oxide	Onyx Oil and Chemical Co.
RO-G	Cetyldimethylamine	Onyx Oil and Chemical Co.
TETROSAN	Alkyldimethyl-3,4-dichlorbenzyl ammonium chloride	Onyx Oil and Chemical Co.
FUNJOL	Alkyldimethyl-3,4-dichlorbenzyl ammonium chloride	Gland-O-Lac Co.
CETAB	Cetyltrimethylammonium bromide	Rhodes Chemical Co.
CETAVLON	Cetyltrimethylammonium bromide	Rhodes Chemical Co.
CTAB	Cetyltrimethylammonium bromide	J. T. Baker Chemical Co.
ETHYL CETAB	Cetyldimethylethylammonium bromide	Rhodes Chemical Co.
OCTAB	Octadecyldimethylbenzylammonium bromide	Rhodes Chemical Co.
ETHYL DECAB	Octadecenyldimethylethylammonium bromide	Rhodes Chemical Co.
OCTIMET	Octadecyldimethylethylammonium bromide	Rhodes Chemical Co.

Trade Name	*Chemical Name*	*Distributor or Manufacturer*
Triton K-12	Cetyldimethylbenzylammonium chloride	Rohm and Haas Co.
Cetyl Zephiran	Cetyldimethylbenzylammonium chloride	Winthrop-Stearns, Inc.
Triton K-60	Lauryldimethylbenzylammonium chloride	Rohm and Haas Co.
Hydrocide	Alkylhydroxybenzyldimethylammonium phosphate	Rohm and Haas Co.
Hyamine 10X	Diisobutylcresoxyethoxyethyldimethylbenzylammonium chloride	Rohm and Haas Co.
Diapene	Diisobutylcresoxyethoxyethyldimethylbenzylammonium chloride	Homemakers Products Corp.
Ceepryn	Cetylpyridinium chloride	Wm. S. Merrell Co.
Fixanol	Cetylpyridinium bromide	E. I. dupont de Nemours and Co.
Retarder LA	Stearyltrimethylammonium bromide	E. I. dupont de Nemours and Co.
In-4276	Decamethylene-*bis* (decyldimethyl ammonium) bromide	E. I. dupont de Nemours and Co.
Septigard-20	Trialkylbenzylammonium chloride	Thompson-Hayward Chemical Co.
Aersol M	Myristamidopropyldimethylbenzyl ammonium chloride	American Cyanamid Co.
Amicide	Alkenyl quaternary ammonium halide	Pioneer Division — The Flintkote Co.
Fuldicide No. 25	Laurylpyridinium chloride	Fuld Bros.
Fuldicide No. 10	Laurylpyridinium chloride	Fuld Bros.
Isothan Q4	Laurylpyridinium chloride	Onyx Oil and Chemical Co.
Quatresin	Myristyl-γ-picolinium chloride	Upjohn Co.
Damol	N-N-N'-N'-tetramethyl-N-N'-didodecyl-β-hydroxypropylenediammonium bromide	Winthrop-Stearns, Inc.
Isothan Q-15	Laurylisoquinoline bromide	Onyx Oil and Chemical Co.
Quartamon	Chlorbenzylate of higher alkylamide of dimethylamino acetic acid	Onyx Oil and Chemical Co.
Q. A. S.	Lauryldimethylbenzylammonium chloride	Mathiesen Alkali Co.

MISCELLANEOUS

Trade Name	*Chemical Name*	*Distributor or Manufacturer*
Duponol C	Sodium lauryl sulfate	E. I. dupont de Nemours and Co.
Duponol WA	Lauryl alcohol sulfate	E. I. dupont de Nemours and Co.
Santomerse D	Alkyl aryl sulfate	Monsanto Chemical Co.
Nacconol NR	Alkyl aryl sulfate	National Aniline Co. (Allied Chemical and Dye Corp.)

Trade Name	*Chemical Name*	*Distributor or Manufacturer*
TERGITOL 7	Sodium sulfate of 3,9-diethyltridecanol-6	Carbide and Carbon Co.
TRITON X-100 (N-100)	Alkylated aryl polyether alcohol	Rohm and Haas Co.
TRITON X-200	Sulfonated alkyl aryl polyether alcohol	Rohm and Haas Co.
TRITON X-500 (W-30)	Sodium salt of aryl alkylether sulfate	Rohm and Haas Co.
TAMOL N	Sodium salt of a condensed aryl sulfonic acid	Rohm and Haas Co.
AEROSOL OT	Dioctyl ester of sodium sulfosuccinic acid	American Cyanamid Co.
TWEEN 80	A polyethylene derivative of sorbitan monooleate	Atlas Powder Co.
METAPHEN	Sodium 4-nitroanhydrohydroxymercuriorthocresol	Abbott Laboratories
MERTHIOLATE	Sodium ethylmercurithiosalicylate	Eli Lilly and Co.
MERCUROCHROME	Disodium salt of 2,7-dibrom-4-hydroxy-mercurifluorescein	Hynson, Wescott, and Dunning
G-11	2,2′dihydroxy-3,5,6,3′,5′,6′-hexachlorodiphenylmethane	Givaudan-Delawanna Co.

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